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A STUDY OF THE GRANULAR AND FLOCCULAR TYPES OF AGGLUTINATION WITH *B. TYPHOSUS**†

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ALTHOUGH the agglutination test with *B. typhosus* is among the oldest and most universal of laboratory procedures for demonstrating evidence of infectious disease, a standardized technic which meets all needs has still to be devised. The inadequacies of the classical microscopic test are obvious. The use of living culture introduces a variable which can be controlled only within relatively wide limits. Moreover, recent investigation indicates that an analysis of certain of the agglutinative properties demonstrable with killed suspensions may furnish information of greater diagnostic significance than can be secured from the results of a single test with a living culture.

Two types of agglutinative properties in serum were demonstrated in 1903 by Smith and Reagh[1] while they were working with motile and nonmotile strains of the hog cholera bacillus. In performing agglutination tests with serum produced with the motile form, they observed a "fluffy precipitate" of the homologous strain and a "granular precipitate" of the nonmotile form which occurred only in relatively high concentrations of the serum, while both strains were agglutinated in a granular manner and to practically the same degree in serum produced with the nonmotile form. Hence, they concluded that the flagella stimulated the production of one type of agglutinative property and the body of the microorganism stimulated the production of another type. Weil

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and Felix,[2] in 1917, reported differences in the character of agglutination obtained with *B. proteus* X 19 in sera from animals artificially immunized with this microorganism and sera from patients having typhus fever. Similarly, they were able to distinguish two types of agglutination obtained with cultures from "Hauch" and "ohne Hauch" colonies of *B. proteus* X 19; hence, they used the initials "H" and "O," respectively, for designating these two types of colony. These letters were later applied to the character of the agglutination obtained with the respective cultures and to properties in sera giving rise to the two forms of reaction. These types of clumping have also been designated as coarse and fine, and as large and small. The terms "floccular" and "granular" describe so well the character of the clumping and correspond so closely to the original terminology employed by Smith and Reagh[1] that their exclusive use seems desirable, not only for purposes of clarity and uniformity, but also in recognition of the priority of these authors' observations. The observations of Weil and Felix[2] and those of later investigators, notably Büchner and Zorn,[3] indicate that the properties in sera which give rise to the granular type of agglutination are heat-labile, while those inducing floccular agglutination are relatively heat-stabile. On the other hand, the corresponding properties in the bacteria are found to behave in an exactly opposite manner, the former being less sensitive to heat than the latter. Further studies, especially those of Weil and Felix[4, 5] and of Weil, Felix and Mitzenmacher,[6] demonstrated similar properties in typhoid bacilli and other microorganisms of enteric disease. Certain species, particularly *B. typhosus* and *B. enteritidis* Gärtner, were shown to have a common factor demonstrated by a granular type of agglutination, while the floccular agglutination was thought to indicate a species-specific reaction. Felix,[7] in 1924, reported that the granular or group-agglutinative property may appear in the sera of typhoid fever patients earlier in the disease than the floccular or specific property and indicates a favorable prognosis. In this investigation, Felix employed living cultures, i.e., one strain of *B. typhosus* particularly rich in the group factor, a second showing both factors in a normal manner, and a strain of *B. enteritidis* Gärtner as a control for the granular type of agglutination.

Felix and Olitzki,[8] in 1928, studied the characteristics of killed suspensions of *B. typhosus* and related microorganisms and reported that low concentrations of phenol and formalin definitely inhibited the granular but not the floccular type of agglutination. On the other hand, they found suspensions treated with alcohol by the method of Bien and Sontag[9] satisfactory for demonstrating the former but not the latter type of agglutination. The observations of these workers indicate that a reaction with a formalin- or phenol-treated suspension of typhoid bacilli furnishes evidence that the patient has typhoid fever, that he has had such an illness in the past, or that he has been inoculated with typhoid vaccine, while a reaction with a suspension treated with alcohol indicates that the patient has an infection due either to the species of bacteria used in the test or to one which is allied to it. Burnet,[10] in 1924, studying a limited number of cases, confirmed the observations of Felix with respect to the sera of typhoid fever patients and of persons who had received typhoid vaccine. Verzár,[11] in 1924, reported the alcohol-treated suspensions of *B. typhosus* and related micro-

organisms to be more sensitive in agglutination tests than those treated with phenol. Gardner[12] and Whitehead[13] agree in general with the findings of Felix and his coworkers, but believe that agglutination with the alcohol-treated antigen may often be demonstrated, in low dilutions at least, in the sera of persons who have received typhoid vaccine and in those of certain normal individuals as well. Felix[14] reports that floccular agglutination does not occur in normal serum, whereas the granular type of agglutinative property may be demonstrated in dilutions as high as 1:100. Craigie,[15] who made an exhaustive study of flagellar and somatic suspensions of the typhoid bacillus, confirms the conclusions of previous investigators that two types of agglutinative property exist, one acting on the flagella and the other on the somata. He also demonstrated very clearly that differences in the macroscopic appearance of the granular and floccular types of agglutination are associated with morphologic properties. A recent report by Eldering and Larkum,[16] indicates that they have found the employment of two antigens of practical value in the examination of sera for evidence of typhoid fever.

For several years, we have hoped to be able to use killed microorganisms in the agglutination test for evidence of typhoid fever. In 1922, Gilbert and Moore[17] reported failure to obtain satisfactory results with formalinized cultures of *B. typhosus* in microscopic agglutination tests. Subsequently, an extensive study of formalin-treated suspensions in macroscopic tests demonstrated that occasionally the sera from patients with typhoid fever which react in the test with living culture do not agglutinate such antigens. Most encouraging results, however, have been obtained in a series of macroscopic tests in which, in addition to a suspension of microorganisms killed with formalin, one treated with alcohol, as outlined by Bien,[18] has been employed. A strain (No. 305) of *B. typhosus* isolated in October, 1929, from a specimen of feces received from a typhoid carrier was found to be suitable for the preparation of these antigens; a nonmotile variant has proved especially satisfactory for that treated with alcohol. Three other strains of *B. typhosus* which have been maintained for a number of years in the bacterial collection, No. 21 Bender, No. 62 Rawlins, and No. 176 Pfeiffer, were found unsuitable for the preparation of the alcohol-treated suspension.

Our experience has indicated that both types of antigen remain satisfactory for at least six months.

TECHNIC USED IN THE MACROSCOPIC AGGLUTINATION TEST

Before the antigens are prepared, the cultures are plated to insure the use of strains which have little, if any, tendency to develop "rough" colonies. Should such colonies be found, "smooth" colonies are fished and replated, a process which may need to be repeated several times. The culture for the formalin-treated antigen should be actively motile.

Formalin-Treated Antigen.—The twenty-four-hour growth from beef-infusion agar in a pint Blake bottle is suspended in from 20 to 30 c.c. of 0.85 per cent salt solution containing 0.2 per cent formalin. After seventy-two hours in the refrigerator, tests for bacterial growth are made and, if necessary, more

formalinized salt solution is added. After the microorganisms have been killed, the turbidity is adjusted to correspond to barium sulphate standard No. 3[19] by the addition of salt solution containing 0.2 per cent formalin, and the agglutinability of the suspension is tested.

Alcohol-Treated Antigen.[18]—The twenty-four-hour growth from beef-infusion agar in a pint Blake bottle is suspended in 10 c.c. of 0.85 per cent salt solution containing 0.5 per cent phenol. The growth from several bottles is combined and one-half the volume of absolute alcohol or a proportional amount of 95 per cent alcohol is added slowly, while the suspension is constantly stirred. It is then allowed to remain at a temperature of from 35 to 37° C. for about eighteen hours after which the supernatant fluid is decanted and tested for bacterial growth and agglutinability. After determination of the dilution necessary to secure a density equivalent to that of barium sulphate standard No. 3, sufficient alcohol is added to the concentrated suspension to give 2.5 per cent in the diluted antigen, which should contain not more than 0.04 per cent phenol.

Equal amounts (0.3 c.c.) of the suspensions, diluted to correspond in density with barium sulphate standard No. 3, and serum dilutions of 1 to 10, 20, 40, 80, and 160 are combined and incubated at from 50 to 55° C. for from eighteen to twenty hours. Clear supernatant fluid and, after gentle shaking, clumps which are definitely discernible to the unaided eye constitute a characteristic reaction. Vigorous shaking should be avoided since clumps of the floccular type are easily dispersed.

TECHNIC USED IN THE MICROSCOPIC AGGLUTINATION TEST

A two- to four-hour culture in bile peptone solution[20] is employed, the density of which is approximately one-half that of barium sulphate standard No. 1.[19] One loopful of each of the serum dilutions used in the macroscopic test is combined with one loopful of culture on cover slips, which are inverted over vaseline-ringed hollow-ground slides. The tests are incubated for one hour at from 35 to 37° C. and the reactions read with a microscope, either a No. 6 or a No. 7 objective being used. Almost complete loss of motility and clumping of the majority of the microorganisms is regarded as characteristic agglutination.

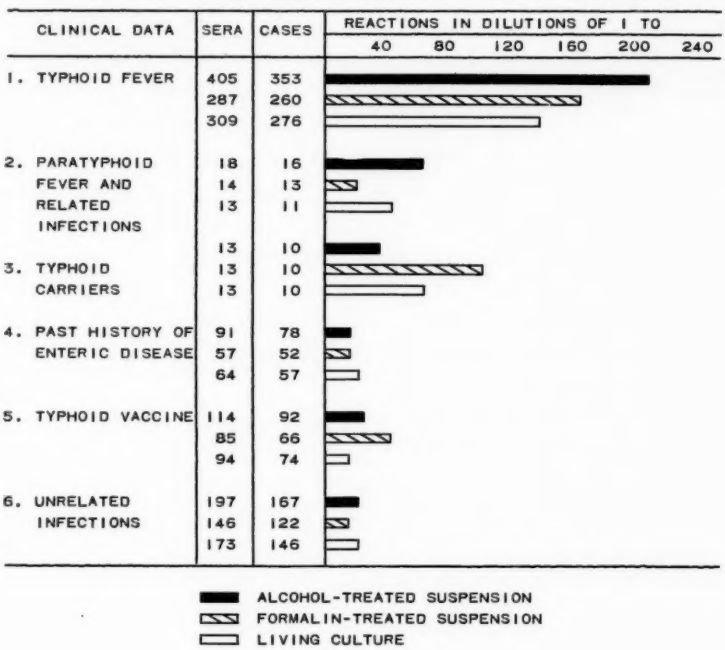
It has been customary to consider of diagnostic significance definite agglutination reactions in the 1:40 dilution in the microscopic test and only those in the 1:80 or higher dilution in the macroscopic test.

Since this study was undertaken, approximately 4,000 sera have been examined. As the use of the various antigens was not begun simultaneously, the number of sera tested with each differs. The specimens have been divided into six groups, according to the clinical data which accompanied them. In the determination of the average agglutination titers, reactions in dilutions higher than 1:320 have not been considered. Only the granular type of agglutination has been observed with the alcohol-treated suspension. Usually the suspension treated with formalin has reacted in a floccular manner, but occasionally, especially in sera from patients having infections with organisms of the paratyphoid-enteritidis group, granular clumping has also been noted.

In the first series, in Chart 1, which represents either clinical or bacteriologically proved cases of typhoid fever, the average titer with the alcohol-treated

antigen is appreciably higher than that with the other two. Of 287 sera tested with all three antigens, 259, or 90 per cent, gave reactions considered of diagnostic significance with the alcohol-treated suspension, and 252, or 87 per cent, with the living culture, while only 220, or 76 per cent, definitely agglutinated the formalin-treated antigen. Twenty-one gave no more than a partial reaction with any of the antigens, but of these, 9 were collected within the first week of illness, while in one instance the duration of illness could not be determined. Two of those giving little or no agglutination, which were collected during relapses from two to two and one-half months after the onset of illness, seem worthy of note. *B. typhosus* was isolated from the clot of one and in both instances blood specimens submitted within the first three weeks after onset gave definite agglutination. One of these cases proved fatal.

CHART 1. AVERAGE AGGLUTINATION TITERS WITH B. TYPHOSUS.



The reactions obtained in infections with microorganisms of the paratyphoid-enteritidis group, although only sixteen cases were studied, correspond to the findings reported. The average titer with the alcohol-treated suspension is more than three times that with the formalin-treated antigen. Five sera from two cases of paratyphoid fever are not included in this group because the patients had had typhoid vaccine. The average titer of these sera with the formalin-treated antigen was only slightly lower than that with the alcohol-treated suspension.

The remainder of the specimens were from patients who were not reported to be suffering from enteric disease at the time of the examination. The fact that only partial reactions were obtained in four sera from three of ten typhoid carriers, substantiates previous observations[21] that the blood of these individuals

does not always give the characteristic agglutination reaction with typhoid bacilli. The floccular agglutinative properties were stronger with the specimens in this group, whereas in Group 4, which includes sera from 78 individuals who had previously had enteric disease but had not become carriers, the average agglutination titer was low and approximately the same with the three antigens tested. Eighty-five sera from 68 individuals who had had vaccine were tested with both antigens, and 24, or 28 per cent, gave reactions in higher dilutions with that treated with formalin. On the other hand, 16, or 18 per cent, agglutinated the alcohol-treated suspension in higher dilutions. In the remaining 45 sera, agglutination either failed to occur or was obtained in the same dilution with both antigens. Very interesting results were obtained with the serum from a woman who stated that she had not had enteric disease, but had received typhoid vaccine one month previously. The formalin-treated antigen was agglutinated in a 1:1,280 dilution, while a partial reaction with the antigen treated with alcohol occurred in a 1:20 dilution only.

The sixth group represents specimens from persons having infections other than enteric diseases, mostly undulant fever, and only those are included which have given some degree of agglutination with one or more of the antigens. Only seven of 197 sera tested with the alcohol-treated suspension and three of 146 tested with the formalin-treated suspension gave definite agglutination, while in the microscopic test, significant reactions were obtained in 42 of 173 sera tested by this method. Over 500 sera from cases diagnosed as undulant fever, which were examined during this period, failed to agglutinate *B. typhosus*. In a previous study,[22] specimens from a considerable number of patients with a febrile disease other than typhoid fever were found to agglutinate the living culture of *B. typhosus* in the microscopic test.

Of the 4,000 sera examined, 106, with which reactions of diagnostic significance were obtained with one or more of the antigens, could not be included in any of the groups, or were accompanied by insufficient information to permit classification. Only 15 of these 106 specimens, however, agglutinated the formalin-treated suspension and only 24 the alcohol-treated suspension, whereas 92 of them reacted with the living culture.

SUMMARY AND CONCLUSIONS

Our study of a series of 4,000 sera has shown that the employment of macroscopic tests with two killed suspensions of typhoid bacilli, one to demonstrate the floccular or species-specific, and the other, the granular or group-agglutinative properties, usually furnishes information of greater diagnostic significance than does the microscopic test with living culture. These findings are in general accord with the results reported by other workers. Apparently, agglutination in a 1:80 or higher dilution with an alcohol-treated suspension usually indicates that the patient has typhoid fever or an infection incited by a species allied to *B. typhosus*, while a similar reaction with a formalin-treated suspension suggests one of three alternatives, that the patient has typhoid fever, has had the disease in the past, or has received typhoid vaccine. Both the granular and floccular types of agglutination have seldom been observed in high dilutions of sera other than those from typhoid fever patients.

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A STUDY OF QUINIDIN EFFECTS ON AMBULATORY PATIENTS WITH AURICULAR FIBRILLATION*

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THE value of quinidin therapy for ambulatory patients who have auricular fibrillation deserves wider recognition. Statistical surveys on the value of quinidin are devoted almost exclusively to usage of large doses on hospitalized patients. Weisman,† however, has pointed out recently the safety and effectiveness of the drug for the ambulatory patient. This study, completed before Weisman's work was published, was made to see if small doses had a substantial effect in converting auricular fibrillation to a sinus mechanism. Twenty-eight ambulatory patients who attended the cardiac out-patient clinic of Northwestern University were the subjects. They received quinidin sulphate in divided doses which totaled no more than 12 gr. (0.8 gm.) daily. Digitalis was also used in most cases simultaneously. Twelve or 44 per cent were restored to sinus mechanism. This result compares favorably with the reports of others who have employed large doses. To our knowledge no patient was harmed by the drug.

TABLE I

CASES RESTORED TO SINUS RHYTHM

NAME	AGE	ETIOLOGIC DIAGNOSIS	PROBABLE DURATION OF FILBRILLATION	PERIOD OF QUINIDIN THERAPY BEFORE SINUS RHYTHM	REMARKS
H. A.	29	Rheumatic	12 mo.	1 mo.	
C. V.	60	Arteriosclerosis	36 mo.	4 mo.	
T. C.	61	Arteriosclerosis	24 mo.	7 days	
S. B.	37	Syphilis	48 mo.	14 days	
J. R.	39	Rheumatic	1 mo.	7 days	
M. M.	57	Arteriosclerosis	30 mo.	7 mo.	
C. M.	47	Rheumatic? Arteriosclerosis	12 mo.	3 mo.	
G. G.	63	Arteriosclerosis	24 mo.	3 mo.	
L. V.	61	Arteriosclerosis	12 mo.	14 days	
C. T.	52	Arteriosclerosis? Thyroid?	8 mo.	2 mo.	
C. L.	29	Rheumatic	48 mo.	1 day	
N. R.	60	Arteriosclerosis	36 mo.	2 mo.	Died later of congestive failure
Average	50	Rheumatic 25% Arterio. 50% Syphilis 8% Doubtful 17%	24 mo.	2 mo.	

*From Cardiac Clinic of Northwestern University.
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†Weisman, S. A.: Auricular Fibrillation (Ambulatory Treatment with Quinidin), Arch. Int. Med. 49: 728, 1932.

An analysis of Tables I and II reveals similar age and similar duration of fibrillation in the restored and nonrestored groups. A larger proportion of restorations occurred in patients with arteriosclerosis as the etiologic factor. Table I shows that 7 of the 12 restored cases had taken quinidin for two months or longer before restoration was effected. From the well-known fact that quinidin is quickly and totally excreted, it is difficult to explain this apparent cumulative effect. Nor can the short period of the slowing of the auricular oscillation rate,* which occurred in this series, throw any light on

TABLE II

CASES NOT RESTORED TO SINUS RHYTHM

NAME	AGE	ETIOLOGIC DIAGNOSIS	PROBABLE DURATION OF FIBRILLATION	PERIOD OF QUINIDIN THERAPY TO 1-12-32	REMARKS
R. A.	48	Rheumatic	33 mo.	14 mo.	
F. G.	44	Rheumatic	13 mo.	1 mo.	
H. B.	51	Arteriosclerosis	50 mo.	14 mo.	
F. S.	62	Rheumatic and syphilis	16 mo.	14 mo.	
S. K.	42	Rheumatic	48 mo.	3 mo.	
M. B.	66	Arteriosclerosis	3 mo.	1 wk.	
R. P.	55	Arteriosclerosis	9 mo.	5 mo.	
J. M.	53	Rheumatic	47 mo.	11 mo.	
G. W.	33	Rheumatic	18 mo.	?	Poor cooperation
A. W.	60	Arteriosclerosis	24 mo.	2 mo.	Refused to continue
I. K.	72	Arteriosclerosis	8 mo.	2 wk.	Refused to continue
J. M.	20	Rheumatic	15 mo.	12 mo.	Death
N. R.	64	Arteriosclerosis	?	2 mo.	Failed to return
M. B.	49	Rheumatic	24 mo.	6 mo.	
F. B.	43	Unknown rheumatic?	?	5 mo.	Failed to return
H. S.	28	Rheumatic	8 mo.	?	Failed to return
Average	49	Rheumatic 50% Arterio. 37% Doubtful 13%	22 mo.	7 mo.	

the mechanism. It therefore seems important to emphasize the value of continuing the administration of small nontoxic doses of quinidin for long periods of time.

SUMMARY

Small doses of quinidin sulphate are capable of establishing a sinus mechanism in a substantial percentage of patients with auricular fibrillation. There is relatively little danger in employing small doses for ambulatory patients. In some patients the drug was not effective until it had been taken for many weeks. Digitalis was used simultaneously and apparently did not interfere with the quinidin action. Small doses of quinidin were capable of slowing the auricular oscillation rate.

*The oscillation rate of auricle was obtained from needle electrodes on the anterior chest wall on 10 patients before and at 15-minute intervals after taking 3 grains quinidin. Seven of 10 showed definite slowing of auricular rate.

THE TREATMENT OF IRRITABLE COLON WITH SODIUM RICINOLEATE*†

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LARSON[1] showed that bacterial toxins could be neutralized by sodium ricinoleate in vitro. Later Dorst demonstrated that certain of the intestinal flora to which patients were highly sensitized could be detoxified in vitro and Morris and Dorst[2, 3, 4] conceived the idea that these toxins might also be neutralized in vivo. They treated cases of irritable colon (mucous colitis) with daily doses of castor oil and later with sodium ricinoleate in gelatin capsules (soricin), obtaining very encouraging results.

They found that their patients were nearly all sensitive to autogenous vaccines made from the so-called "normal flora" of the intestinal tract, according to the method of Wherry.[5] They also found that these vaccines could be detoxified with sodium ricinoleate, so that at least three times as much could be given without undue reaction. Detoxification did not weaken the antigenic powers. They demonstrated that, by giving these patients sodium ricinoleate orally, the skin sensitivity to heat-killed bacterial vaccines was diminished.

The purpose of this paper is to determine the effects of soricin on the symptoms and skin sensitivity to autogenous stool vaccines in cases of irritable colon. The patients were selected in the Out-Patient Dispensary and all attempts made to rule out coexisting disease, particularly intraabdominal. A diagnosis of irritable colon (or mucous colitis) was based on the accepted criteria of a history of abdominal pain, distention, constipation, a passage of excess flatus, borborygmus, nervousness, often the presence of mucus in the stools, the presence of a palpable and tender colon, and x-ray evidence of spasm.

Cultures of the stools of each patient were made. The individual strains of organisms were isolated and suspended in salt solution. They were then killed by heating to 56° for one hour. One minim of each suspension was injected intradermally and the local reaction observed twenty-four hours later. Treatment with soricin was then instituted for various periods of time.

The doses varied from 5 to 30 gr. 4 times daily. Soricin was well tolerated by a great majority of the patients. The few complaints made dealt with the large size of the capsules, occasional "sour belching," and a slight burning sensation in the epigastrium. The capsules were administered before meals and at bedtime.

*From the Department of Internal Medicine, University of Cincinnati.
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†This work was made possible through a grant by the Wm. S. Merrell Company, Cincinnati, Ohio.

After a period of treatment, generally of two to three months' duration, the skin tests were repeated with the original suspension of organisms. Age did not diminish the intensity of the reaction to the vaccine, as was determined by comparing the effects of a newly made autogenous vaccine with a preparation a few months old, containing the same strains of organisms.

CASE 1.—A white man, aged twenty-seven, single. The patient was first seen on April 4, 1932, complaining of gas on the stomach and pain in the abdomen. During the four weeks before admission he had noticed a dull aching pain localized below the xiphoid process, coming on three or four hours after eating and lasting until he would take soda or food. Belching gave temporary relief. He had a similar attack one year previously, lasting four weeks. He was frequently constipated.

There was localized tenderness in the epigastrium just below the xiphoid process. The abdominal findings were not otherwise remarkable. A gastric analysis following ingestion of an Ewald meal revealed free HCl 77, total acids 102. The gallbladder visualized normally. Gastrointestinal x-ray studies showed spasm of the transverse and descending colon with mucus present in the latter. He was given soricin, grains 10, four times a day. Three weeks later this was increased to 20 gr. and continued until June 9, 1932.

The appetite increased, the amount of belching and flatus were definitely diminished. The pain was very much lessened and disappeared entirely in six weeks. The constipation was relieved by liquid petrolatum. He still complained of nervousness. There was no change in weight.

The organisms recovered from the stools and results obtained were as follows:

VACCINES	MAY 23, 1932	JUNE 28, 1932
1. Hemolytic bacillus coli	3.5 x 6 cm.	negative
2. Nonhemolytic bacillus coli	negative	negative
3. *Streptococcus viridans*	1.5 x 2 cm.	negative
4. *Streptococcus hemolyticus*	negative	negative
5. *Staphylococcus albus*	negative	negative

CASE 2.—A white man, aged forty-four, single, vocation electrician. The patient was first seen in October, 1929, complaining of abdominal discomfort, fullness and belching about one to two hours after meals and constipation for a period of ten years. He also complained of nervousness. His appetite was fair. There was some palpitation of the heart occasionally after meals.

Physical examination revealed tenderness on deep pressure to the right of the midline in the epigastrium and to the right of the umbilicus. The liver was felt 2 fingerbreadths below the right costal margin. Gastrointestinal x-ray series indicated the presence of mucus in the colon. Gastric analysis after ingestion of an Ewald meal revealed free HCl 35, total 59. The patient was placed on a smooth diet with bromide, belladonna, and cascara and obtained great relief from the above symptoms until April 4, 1930. He was not seen again until Dec. 2, 1930. He felt well until two months before that time, when his former symptoms returned and the presence of mucus was noticed in the stools. Physical examination showed no changes except an occasional premature ventricular contraction. The same treatment was started again, but he failed to improve. On Jan. 8, 1932, he was placed on soricin, 5 gr. 4 times a day and bromides, belladonna, and soda after each meal. From that time until May 18, 1932, his medications were changed so that for two or three weeks he would receive only soricin, 10 gr. 4 times a day, then only bromide, belladonna, and soda for a similar period, then both together. He received almost complete relief from epigastric pain and belching, and the amount of flatus was reduced while taking soricin alone. Constipation was not relieved except when taking some laxative and mucus was present in the stools from time to time. This patient had frequent head colds during the treatment and found his symptoms aggravated each time.

Organisms isolated from the stools were made into vaccines and the patient was skin-tested with each vaccine. The organisms recovered and the results obtained were as follows:

VACCINES	APRIL 17, 1932	JUNE 27, 1932
1. *Bacillus coli*	3.5 x 5 cm.	1.5 x 1.5 cm.
2. Hemolytic streptococcus	negative	negative
3. *Streptococcus viridans*	negative	negative

CASE 3.—A white man, aged forty, married, vocation, motorman. The patient was first seen on Dec. 8, 1931, with the following complaints, which he said had been present for five years: Constipation, passage of much flatus, some bloating and belching. The stools were hard and lumpy, but contained no mucus. He complained of much nervousness and frequent headaches. He came to the clinic because he thought he had cancer.

Physical examination revealed dental caries. There was slight thyroid enlargement with normal blood pressure and pulse rate. The abdomen was negative. Gastrointestinal series showed only spasticity of the descending colon with the presence of mucus. Gastric analysis after ingestion of an Ewald meal revealed free HCl 22, total 38. The patient was placed on tincture of hyoscyamus and sodium bromide and tincture of belladonna, liquid petrolatum and a smooth diet. He felt quite well for two weeks on the above regime. He was then given soricin, 5 gr. 4 times a day with no other medication. The dose of soricin was gradually increased to 10 gr. 15 gr., and 20 gr. 4 times a day. This treatment was continued until June 15, 1932. The effect of soricin upon his symptoms may be expressed as follows: There was no change in the amount of belching, he did not complain of pain at any time, and there was no change in the amount of flatus. He still complained of constipation unless liquid petrolatum was taken regularly. No mucus was seen at any time. Nervousness was somewhat lessened, and he found that he could eat some foods without discomfort which previously he could not tolerate. Organisms isolated from the stool were made into vaccines and the patient was skin tested with each vaccine.

VACCINES	MAR. 18, 1932	MAY 23, 1932
1. Gram-negative bacillus (not an acid producer)	11.5 x 17 cm.	8 x 11 cm.
2. Colon bacillus	11.5 x 17 cm.	5.5 x 6.5 cm.
3. Staphylococcus	negative	2.5 x 3.5 cm.

Twenty-four hours after the injection of the vaccines on Mar. 18, 1932, the reacting areas in Nos. 1 and 2 were red, swollen, with increased local heat. There were 2 red streaks running up the inner aspect of the arm toward the axilla, and there was tenderness in the axilla. The patient had a temperature of 100.5° F. and complained of a headache and backache and general malaise. It was one week before the reactions disappeared from his arm. At the second test the areas of redness were very definite, but there was no generalized reaction.

CASE 4.—A white man, aged thirty-five, vocation, policeman. The patient was admitted to the Cincinnati General Hospital on Jan. 5, 1932, complaining of pain and a heavy feeling in the lower abdomen. During the past four years he had noticed a dull aching pain just below the umbilicus, occasionally radiating to the back. It was not related to meals and was not relieved by eating or drinking. At times it would become severe but never agonizing. His appetite was good, there was frequent belching, no nausea or vomiting. His bowels moved once a day, but in addition he complained of a desire to pass stools at other times without effect. He noticed mucus in his stools frequently. He complained of a tired aching feeling most of the time and of frequent "nervous spells." Three years ago his appendix was removed but very little relief from the above symptoms was obtained. The history was otherwise negative.

Physical examination: The patient was a well-developed but somewhat undernourished white man, appearing chronically ill. The abdominal examination revealed slight tenderness

just below the umbilicus. No masses or solid organs were felt. The colon was not palpable. The red blood count was 3,100,000, hemoglobin 65 per cent (Sahli). The urine was essentially normal. The gastric analysis following the ingestion of an Ewald meal revealed free HCl 6, total 21. An oatmeal gruel fractional test meal revealed normal acidity. Stool examination was essentially negative. Basal metabolic rate was –8 and–4 per cent. Gastrointestinal series showed areas of spasm in the transverse and descending colon. A barium enema showed spasticity of the terminal third of the colon and incompetence of the ileocecal valve. The patient was placed on soricin, 10 gr. 4 times a day, starting Jan. 15, 1932. One week later this was increased to 20 gr. 4 times a day and two weeks later it was increased to 30 gr. 4 times a day and that dosage continued, except as will be described later, until June 15, 1932. He obtained very little relief from his symptoms during the first three weeks, but he then was placed on tincture of hyoscyamus, sodium bromide and tincture of belladonna in addition to the soricin for a period of two months. Soricin was discontinued during the following three weeks but was started at the end of that time at the request of the patient and the two were used in combination until June 1, 1932. He found that more relief was obtained with a combination of the two than with either one alone. The results attributable to soricin were as follows: His appetite improved. Nausea occurred on several occasions whereas very little had been noticed before treatment was started. The pain was definitely lessened. His bowels moved more regularly while taking soricin. The stools were better formed but possessed a very offensive odor. There was no change in the amount of flatus. Nervousness was definitely lessened, and he found that he could tolerate foods that previously caused him discomfort. There was no change in the weight. Stool cultures were made at the beginning and the organisms recovered were made into vaccines for skin testing. The organisms recovered and the results obtained were as follows:

VACCINES	MAR. 18, 1932	JUNE 6, 1932
1. Colon bacillus	7.5 x 16 cm.	6.5 x 7.5 cm.
2. Gram-negative bacillus (without formation of acids)	7.5 x 16 cm.	5. x 5.5 cm.
3. Streptococcus	negative	negative

Twenty-four hours after the injection of the vaccines on Mar. 18, 1932, the reacting areas of Nos. 1 and 2 were red and swollen with increased local heat. Red streaks were seen running up the inner aspect of the arm toward the axilla. There was a small tender gland palpable in the axilla. He had a slight fever, complained of headache and general malaise. At the second test the areas of redness were very definite but there was no generalized reaction.

CASE 5.—A white man, aged fifty-three, married, vocation, fireman. The patient was first seen on Jan. 3, 1930, complaining of abdominal pain and diarrhea. For two years he had suffered from a gnawing and dragging type of pain over the abdomen. This was not localized and not related to meals. He often complained of headaches over the frontal region, of extreme nervousness and 2 or 3 loose stools a day. He had not noticed blood or mucus in the stools at any time. An appendectomy had been performed ten years before admission. There had been no weight loss. The examination of the abdomen revealed the colon to be palpable and acutely tender when pressure was applied over the lower third. The cecum and ascending colon were palpable. He was not seen again until two months later, at which time he complained of the same symptoms, and in addition he had noticed mucus and blood in the stools on 2 occasions. He had lost 15 pounds in weight. Abdominal examination revealed tenderness in both lower quadrants and in the epigastrium. Gastric analysis following the ingestion of an Ewald meal showed free HCl 38, total 52. Gastrointestinal series was negative, as far as the upper gastroenteric tract was concerned. A barium enema revealed spasticity of the descending colon. Proctoscopic examination showed only 1 small hemorrhoid. He was placed on a smooth (low residue) diet with bromides and belladonna after meals. He continued on this regime with some variations until Sept. 17, 1931. During that time he showed very definite improvement for a few weeks, until the excitement of a big fire or a near accident would set him back. On September 17, he stated

that his appetite was fair, there was some belching and rumbling through the intestines, some flatus and 3 or 4 loose bowel movements every day. He was quite nervous and always tired. Soricin, 5 gr. 4 times a day was given and all other medication and diet discontinued. The soricin was increased to 10 gr. 4 times a day on Oct. 16, 1931, to 15 gr. on Jan. 6, 1932, and to 30 gr. on Jan. 20, 1932, and continued at the latter dosage until April 27, 1932. The only other medication used during that time was occasionally a half grain of luminal at night. Throughout the course of soricin treatment he experienced the same tendency to improve for a time and then a setback would follow some excessive excitement or acute upper respiratory infection. The amount of belching and flatus were increased until the larger dose of soricin was given and then there was a definite decrease. The amount of distention and bloating was somewhat lessened. The number of bowel movements and character of the stools were unchanged. The abdominal pains were slightly diminished at first and after instituting a larger dose they disappeared. There was very little change in the degree of nervousness. No change in weight was found. Organisms isolated from the stools were made into vaccines and used to test the skin sensitivity. The organisms used and the results obtained were as follows:

VACCINES	SEPT. 17, 1931	DEC. 23, 1931
1. Colon bacillus	4+	11 x 5 cm.
2. *Staphylococcus citreous*	1+	negative
3. *Staphylococcus aureus*	1+	negative

The reacting area of the No. 1 on Sept. 17, 1931, was very marked, red, swollen and tender with red streaks up the inner aspect of the arm toward the axilla. No such reaction as this was obtained on Dec. 23, 1931.

CASE 6.—A white man, aged twenty-four, married, vocation, draftsman. He was first seen on Feb. 17, 1931, complaining of "indigestion." For the past three weeks he had noticed a sharp cutting or sticking pain in the left upper quadrant, radiating to the left flank. This would appear during his meals and would last from one-half to two hours. Though his appetite was good there was fear of eating, much belching and flatus, some nausea but no vomiting. His bowels moved regularly; there was no mucus in the stools. Coffee, tea, tobacco, fried and fatty foods caused him discomfort. He had complained of a similar condition one year before. He stated that he had been jaundiced eight years ago.

On examination, the abdomen was found to be below the chest level. There was tenderness in the left upper quadrant. No masses or solid organs were felt. Gastric analysis after ingestion of an Ewald meal revealed free HCl 33, total acids 50. The stomach, duodenum, and gallbladder were entirely negative. Barium enema showed a moderate amount of mucus in the upper part of the descending colon. He was placed on bromides, belladonna and hyoscyamus and a smooth diet with temporary improvement.

He was next seen in the clinic on Jan. 25, 1932, with complaints similar to those on the previous admission.

He was placed on the same regime as before for a period of two months with slight improvement. The treatment was then discontinued and the patient given soricin, 30 gr. 4 times a day. At the end of one week the dosage was cut to 15 gr. 4 times a day and continued at that dose until May 25, 1932, when it was discontinued at the patient's request. He stated that soricin caused a burning sensation in the epigastrium soon after taking it and that the belching, rumbling sounds, and amount of flatus were all increased. He felt that it also caused him to be more constipated and said he felt much better when not taking it.

Organisms were isolated from the stool and made into vaccines. The organisms recovered and the reactions obtained from skin tests were as follows:

VACCINES	APRIL 10, 1932	JUNE 28, 1932
1. Hemolytic colon bacillus	5 x 7 cm.	3 x 4 cm.
2. Nonhemolytic colon bacillus	2.5 x 3 cm.	0.8 x 1.0 cm.
3. Small gram-positive diplococcus	2.5 x 4 cm.	1 x 1.5 cm.
4. Streptococcus	3 x 3.5 cm.	1 x 1.2 cm.

It is interesting to note that in spite of decrease in bacterial sensitivity, no improvement of symptoms occurred. Since that time we have had other capsules prepared with a coating which does not dissolve in the stomach. Better results have been obtained in similar cases with the new capsules.

CASE 7.—A white woman, aged twenty-five, single. This patient was first seen on April 12, 1931, complaining of pain and a feeling of tightness over the abdomen, and constipation. Her appetite was good. There was much belching, some nausea, distention of the abdomen, with rumbling sounds and much flatus. The stools were hard and in small lumps containing mucus. Occasionally she would complain of diarrhea. There were frequent headaches and backaches. She had the above symptoms for over three years before admission to the clinic. One year before the appendix, right ovary, and right tube were removed.

Examination of the abdomen revealed a right rectus scar and tenderness in both lower quadrants. Pelvic examination was negative. Gastric analysis after the ingestion of an Ewald meal revealed no free HCl, total acids 6. Free HCl was present after the injection of ½ mg. of histamine. Gastrointestinal series revealed spasticity of the colon with excess mucus in the sigmoid region.

She was placed on antispasmodics, sedatives, mild laxatives, and a smooth diet. This regime was continued for four months without any noticeable improvement. Soricin, 5 gr. 4 times daily, later increased to 10 gr. 4 times a day was given for two months without improvement. On Jan. 22, 1932, she was placed on soricin, 20 gr. 4 times a day, with liquid petrolatum as a laxative. This was increased in two weeks to 30 gr. and continued until May 18, 1932, when she had an attack of acute catarrhal jaundice. Her appetite remained good throughout. There was no reduction in the amount of belching and flatus. She complained of much abdominal distention and bloating. The gas pains were not diminished in frequency or severity. Soricin had no effect on the constipation. There appeared to be a definite lessening in the amount of mucus. There was no change in weight.

A stool culture in August, 1931, revealed an overwhelming growth of colon bacillus. This was made into a vaccine. A marked reaction was obtained on intradermal injection. Unfortunately, the vaccine was lost so that no test was made after treatment with soricin.

CASE 8.—A white male, aged thirty-two years, single. This patient was admitted to the hospital on Dec. 3, 1930, complaining of nervousness and bowel trouble. These symptoms had been present for thirteen years and during that time the patient had frequently noticed "yellow mucus clots" in the stools. He took a cathartic or an enema almost daily during that time. In 1924, a colostomy was performed and the colon irrigated because of a "mucous colitis." This gave improvement of symptoms at that time but after closure of the wound the same symptoms returned. He was quite excitable and worried a great deal about his condition.

On physical examination the abdomen was negative. Gastric analysis after the ingestion of an Ewald meal gave free HCl 38, total acids 53. Stool examination was negative for mucus, blood, and parasites. The patient was not seen again for a year and returned complaining of the same symptoms. A gastrointestinal x-ray series showed some redundancy of the hepatic flexure and spasticity of the descending colon. Gastric analysis revealed free HCl 48, total acids 70. He was placed on soricin, 5 gr. 4 times a day, until June 1, 1932. His appetite was good throughout. There was no belching, nausea, vomiting, or abdominal pain at any time. Constipation was relieved to some extent by liquid petrolatum, the soricin appearing to make him more constipated. The nervousness was somewhat lessened. There was a loss of 6 pounds in weight. Organisms cultured from the stool were made into vaccines and used to determine the skin sensitivity. The organisms recovered and results obtained were as follows:

VACCINES	MAR. 17, 1932	JUNE 28, 1932
1. Hemolytic colon bacillus	negative	negative
2. Nonhemolytic colon bacillus	5.5 x 6.5 cm.	3 x 4 cm.
3. Streptococcus	negative	negative
4. Staphylococcus	negative	negative

CASE 9.—A white man, aged forty-one years, married. The patient was first seen on Feb. 1, 1932, complaining of constipation. For the past nine years he had been troubled with constipation, passage of mucus in the stools, nausea, belching, and a dull pain under the left costal margin. There was also considerable nervousness and sweating. During the last eight weeks before admission to the clinic all of his symptoms were much intensified.

On physical examination the abdomen was tender in both lower quadrants. The descending colon was palpable and tender. Gastric analysis following the ingestion of an Ewald meal revealed no free HCl. Following an injection of ½ mg. of histamine the free HCl was 62.0. Blood Wassermann was negative. A gastrointestinal x-ray series showed narrowing of the descending colon with absence of the haustra. A barium enema revealed spasticity of the distal third of the colon. The gallbladder visualized normally. A proctoscopic examination revealed only external and internal hemorrhoids. On Feb. 12, 1932, he was placed on bromides, hyoscyamus, belladonna, and soda with liquid petrolatum as a laxative. This regime was continued until April 8, 1932, without noticeable improvement. Soricin, 10 gr. 4 times a day was then added to the above medication, and he continued with that regime until May 27, 1932.

The appetite was unchanged. Belching was lessened but he complained of belching after taking the soricin, stating that the gas had a bad odor. There was definite reduction in the severity of the abdominal pain. There was some reduction in the amount of mucus. Constipation was relieved by liquid petrolatum. He still complained of much nervousness. There was no change in weight.

Organisms isolated from the stool were incorporated into vaccines and used to test the skin sensitivity. The organisms recovered and the results obtained were as follows:

VACCINES	JUNE 4, 1932	JUNE 28, 1932
1. Hemolytic colon bacillus	2.5 x 3.5 cm.	negative
2. Nonhemolytic colon bacillus	2.5 x 3.5 cm.	1.5 x 2 cm.
3. Streptococcus	negative	negative

CASE 10.—A white man, aged twenty-seven, single. This patient was first seen in March, 1929, complaining of pain in the abdomen. This was of a dull aching character located in the right upper and lower quadrants, appearing about an hour and a half after each meal. It was relieved slightly by soda and completely by a bowel movement. The pain was first noticed two years before admission. He was habitually constipated and had noticed mucus in the stools for several years. He had lost 17 pounds in weight.

The physical examination was essentially negative throughout. A gastrointestinal x-ray series was negative. The gallbladder visualized faintly after the oral and intravenous administration of iodeikon. On May 31, 1929, a cholecystectomy and appendectomy were performed following an acute attack of pain in the right upper quadrant. A chronic cholecystitis without stones was found at operation; the appendix was normal.

He was not seen again until Jan. 16, 1931, at which time he complained of the same symptoms as before operation. A gastric analysis after the ingestion of an Ewald meal revealed free HCl 48, total acids 70. A gastrointestinal x-ray series showed only hyperperistalsis. He complained of a dull pain in the right half of the abdomen, much belching and flatus, constipation with the passage of mucus in the stools, and nervousness. Various types of treatment were tried without results. In December, 1931, a series of elimination diets was used to determine the presence of food allergy with negative results. On Jan. 28, 1932, he was placed on soricin, 15 gr. 4 times a day and liquid petrolatum. In two weeks the soricin was increased to 30 gr. 4 times a day and maintained at that dosage until June 1, 1932, with the exception of ten days during May when he was in the hospital for another condition.

The appetite was poor throughout, there was slight lessening in the amount of belching and flatus. The abdominal pain was diminished. No mucus was seen in the stools after treatment with soricin was started. Constipation was controlled with liquid petrolatum. The nervousness was definitely lessened. During the ten days he was in the hospital and not receiving soricin, he stated that his symptoms were much increased in severity.

Stood cultures were made and used to test the skin sensitivity. The organisms used and results obtained were as follows:

VACCINES	MAR. 23, 1932	JUNE 6, 1932
1. *Bacillus mucosus*	4 x 5 cm.	negative
2. *Bacillus coli*	5.5 x 11 cm.	3.5 x 5.5 cm.
3. Staphylococcus	negative	negative
4. Streptococcus	2 x 2 cm.	negative

The reacting areas of Nos. 1 and 2 on Mar. 23, 1932, were red, hot, and swollen, and there was pain in the corresponding axilla. No such reaction was noticed with the second test.

CASE 11.—A white man, aged forty-one, married, vocation, engineer. The patient was first seen on Nov. 24, 1931. For the past five years he had complained of a dull aching pain radiating across the lower abdomen, coming on about one-half hour after each meal and lasting a few minutes, followed by much belching. The appetite was good, there was much rumbling of the intestines. Generalized abdominal soreness and constipation were present. No mucus or blood was seen in the stools. There was a history of syphilis eight years ago, for which he had received active treatment.

Physical examination: On physical examination there was abdominal tenderness, and the descending colon and sigmoid were palpable and tender. X-ray examination revealed moderate spasm of the transverse and descending colon. Gastric analysis following ingestion of an Ewald meal gave free HCl 20, total acid 36.

He was placed on a smooth diet of Alvarez, liquid petrolatum and tincture of belladonna for one month without noticeable improvement. On Dec. 30, 1931, soricin, 5 gr. 4 times a day, was started, all other medications being discontinued. The soricin was increased to 10 gr., then 20 gr., then 30 gr. 4 times a day, allowing two weeks to elapse between each increase, and the latter dosage continued until May 25, 1932. Liquid petrolatum was given as a laxative. Pain was much reduced after the first two weeks' treatment with soricin and was practically absent until May 5, 1932. Since then, it has been present at intervals, though of little consequence. The amount of belching was definitely lessened for four months and then returned. There was some diminution in the amount of flatus. Soricin had no effect on the constipation. His appetite remained the same throughout, but there was a loss of 12 pounds in weight.

Organisms isolated from the patient's stool were made into vaccines and the patient was skin tested. The organisms recovered and results obtained were as follows:

VACCINES	APR. 20, 1932	JUNE 8, 1932
1. *Bacillus coli*	3.5 x 4 cm.	4 x 4 cm.
2. *Staphylococcus albus*	4.5 x 5 cm.	4 x 4.5 cm.
3. Streptococcus	negative	negative

There was no apparent change in the reactions to the vaccines after treatment with soricin for a period of approximately five months.

CASE 12.—A white man, aged twenty-eight, single. The patient was first seen on Mar. 14, 1932, complaining of pains in the abdomen of two months' duration. They were cramp-like in character and at first confined to the epigastrium. Later they began to localize in the right upper quadrant. For two days they were accompanied by nausea and vomiting and aching over the entire body. At times there was some radiation to the right shoulder blade. His appetite was good. There was some fullness after eating during the past three or four months. Tarry foods and fried foods caused discomfort. He has been constipated since the onset of this illness. There has been a loss of 15 to 20 pounds in weight.

On physical examination there was increased resistance over the right half of the abdomen but no tenderness. Gastric analysis after the ingestion of an Ewald meal revealed free HCl 47, total acid 62. Gastrointestinal x-ray studies showed irritability of the duodenum and slight downward displacement of the hepatic flexure of the colon. The gall-

bladder visualized normally. The patient was placed on a bland diet and hyoscyamus and bromides with liquid petrolatum for the constipation. He continued on this regime for five weeks without noticeable improvement. At this time the above medication was discontinued except for the petrolatum, and he was given soricin, 10 gr. 4 times a day. This was increased to 15 gr. and then to 20 gr. 4 times a day. The belching was definitely lessened and he had relief from nausea. There was a marked diminution in the abdominal pain.

The organisms cultured from the stools used and results obtained were as follows:

VACCINES	MAY 28, 1932
1. Hemolytic bacillus coli	3.5 x 6.5 cm.
2. Nonhemolytic bacillus coli	negative
3. *Staphylococcus albus*	negative

The patient did not return to the clinic for a second determination of sensitivity.

SUMMARY

Twelve patients exhibiting the symptoms which are attributed usually to an irritable colon have been treated with sodium ricinoleate. Prior to initiation of this treatment, these patients had failed to respond to a bland diet and antispasmodics. It is to be noted that the cases reported by Dorst and Morris[4] had all been under treatment for a period of six months or longer. Some of the inconclusive results obtained with certain of the cases included in this series are reported after only three months of treatment and would probably respond to persistent therapy. The general results may be outlined as follow:

There was diminution of the pain and belching in the majority of the cases. The amount of flatus was diminished in five. As has been previously shown, constipation was not affected. Mucus in the stools was appreciably lessened or disappeared in 50 per cent of those who showed this symptom. It is important to note that those patients who did not show clinical improvement in the treatment with soricin had, with one exception, definite gastric symptoms following the ingestion of the drug. This demonstrates the necessity for having more adequate enteric coating, since the sodium ricinoleate, if liberated in the stomach and acted upon by gastric secretions, gives rise to distressing symptoms.

The skin sensitivity to organisms from the intestinal flora was reduced materially in 9 out of 10 cases. In two cases it was impossible to obtain sensitivity records at the end of treatment.

CONCLUSIONS

1. It is felt that soricin is of definite benefit in the treatment of irritable colon.

2. There is a decrease in the skin sensitivity to bacterial vaccines following treatment with soricin.

This tends to support the theory of Dorst and Morris, that soricin neutralizes bacterial toxins in vivo.

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THE EFFECT OF SALYRGAN AND X-RAY ON THE RATE OF DISAPPEARANCE OF THROMBOPHLEBITIC EDEMA*

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IN A PREVIOUS publication,[1] we reported a method for producing edema in the hind legs of experimental animals comparable to that seen in man following deep thrombophlebitis. Chemical study of the edema fluid revealed a rather high total protein content, the values ranging from 1.25 to 2.34 per cent. It was indicated that this accumulation of protein-rich fluid in the tissue spaces might be a factor in the perpetuation of postphlebitic indurations.

In possession of a method to produce such an edema, it was possible to evaluate the effect of measures which might hasten the disappearance of the acute edema in order to prevent chronicity. Consequently, the disappearance rate of experimentally produced edema in untreated animals was compared with that in dogs given a mercurial diuretic or x-ray exposure. In the first series of experiments, salyrgan was injected to determine the possibilities of mobilizing the edema fluid by this method.[2] The effect of x-ray was studied because of the excellent results reported from more or less empirical clinical use of such treatment.[3]

METHODS OF EXPERIMENTATION AND RESULTS

Edema was produced in the leg of the dog by the method previously described, namely, the injection of a concentrated tissue extract (Fibrogen, Merrell) peripherally into the femoral vein. This results in extensive peripheral, intravascular thrombosis, and massive edema of the extremity. The dosage of fibrogen varied from ½ to 1 c.c. and the degree of edema was usually roughly parallel to the quantity of fibrogen injected. Careful measurements of the circumferences of the limbs were made before and at varying intervals after the injections. Readings were taken at four levels: through the middle of the thigh, the popliteal fossa, the middle of the leg, and the foot. Subtracting the dimensions before injection from those after it, gave a measure of the increase in circumference due to edema. Because the variations in

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diameter at the thigh and knee greatly exceeded those of the leg and foot, a sum of the four differences, rather than an average, was taken as an index of the amount of edema. The measurements were almost entirely made by the same person, who often was not informed which of the animals were controls, and which experimental. Comparison of the circumferences of the two hind legs of the same animal revealed the margin of error in the measurements to be very small.

CONTROLS

In a series of twenty animals, edema was produced by the method described, and measurements taken at intervals over a period of two weeks. The results are shown in Chart 1. The heavy line represents the average curve in this series illustrating the rate of disappearance of edema in untreated animals. In all other respects these dogs were kept under the same conditions as were the ones in the following groups.

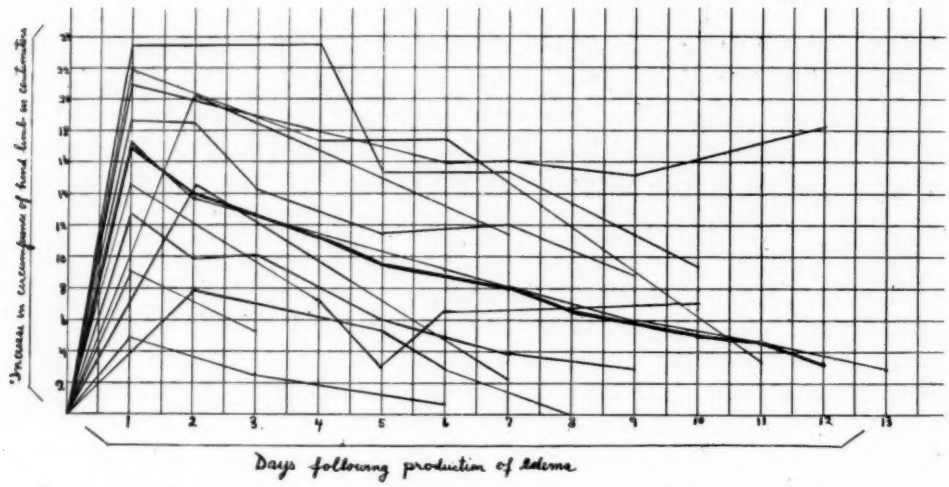

Chart 1.—The normal rate of disappearance of experimentally produced edema. The heavy line represents an average curve of increase and decrease of a hind limb in the untreated animal.

SALYRGAN EXPERIMENTS

In this series of twenty animals edema was produced in exactly the same way as above. At varying intervals after the edema reached its height, salyrgan, in doses of 1 c.c., was injected intravenously into the opposite extremity. In some experiments the injection of salyrgan was repeated. Salyrgan was used alone, without ammonium chloride or other acidifying agent. The results of these experiments are seen in Chart 2. The arrows indicate the time at which the salyrgan was given. The heavy black line represents the composite curve for this series of experiments. It will be seen from these curves that a rapid diminution in the circumferences of the edematous limbs occurred immediately following the salyrgan injection. It is also seen that repetition of the injection usually failed to produce a second sharp reduction in the degree of edema. The disappearance curve of the salyrgan treated animals is super-

imposed upon that of the controls in Chart 4. In this figure the average maximum circumference increase of each series represents 100 per cent, and the curve shows the percentage daily decrease in the respective groups. From this it will be seen that the greatest acceleration of disappearance time following salyrgan administration occurred during the first few days, after which the two curves run an almost parallel course.

It must be stated that no attempt was made to regulate the fluid intake of the experimental animals in this group, because no such restriction had been made in the control series. It is quite likely that, following the diuresis and consequent dehydration which resulted from the salyrgan injection, the animals consumed large quantities of water, and thus, in a measure, replaced the fluids lost. It seems reasonable to suppose that, had the fluid intake been restricted, as has been done in the case of human beings subjected to diuresis for the purpose of mobilizing excessive tissue fluid,[2] the rate of disappearance of the

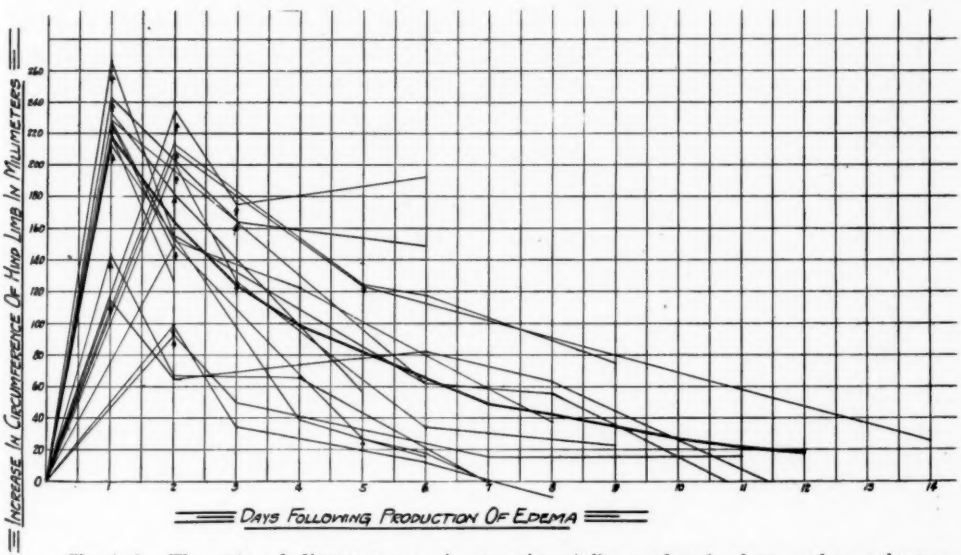

Chart 2.—The rate of disappearance in experimentally produced edema, when salyrgan was injected at the height of the edema. The arrows indicate the injections of salyrgan. The heavy line represents the composite curve for this series. It will be seen, that the injection of salyrgan is followed by a rapid diminution in the circumferences of the edematous limbs. The second injection does not seem to be as efficient as the first.

edema would have been more rapid. These experiments at least serve to indicate that diuresis produced by the administration of mercurial diuretics is capable of mobilizing edema fluid from the extremities of animals in which experimental postphlebitic edema had been produced.

X-RAY EXPERIMENTS

The therapeutic use of x-ray has recently been advocated for the treatment of early edemas of postphlebitic origin, notably by Halban, of Vienna.[3] Excellent results are claimed for this treatment. A series of experiments was performed to determine the relative effect of x-ray upon the rate of disappearance of postphlebitic edema in the laboratory animal. Edema was produced as in the other series, and at the height of the edema, the limbs were exposed

to x-ray in doses varying from 100 to 400 r. Measurements were made of the circumferences before and after irradiation, and the results plotted on Chart 3. From these curves it may be seen that a definite diminution in the edema oc-

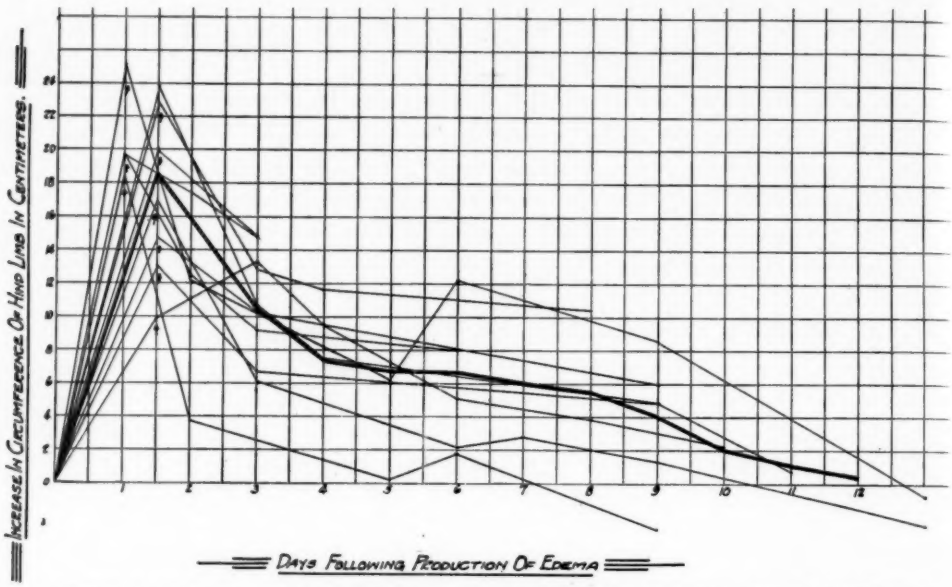

Chart 3.—The rate of disappearance of experimentally produced edema following exposure to roentgen ray. The heavy line represents the average curve. The exposure was not always given at the height of edema.

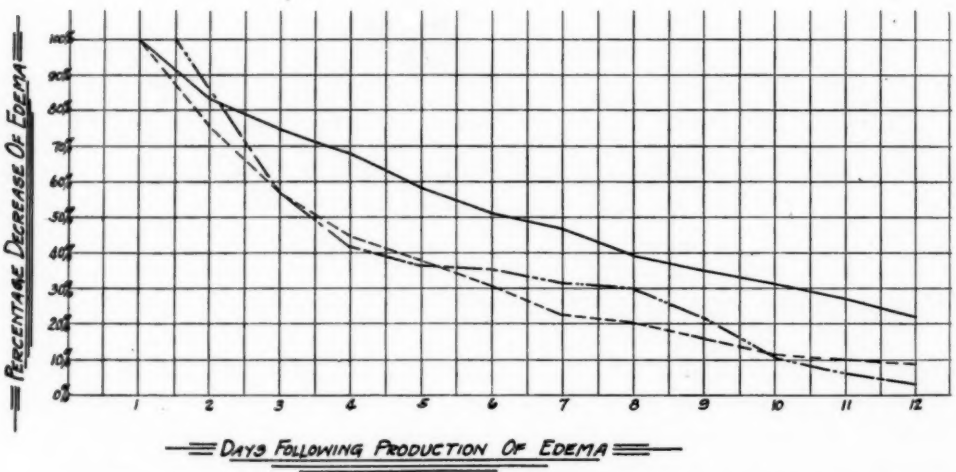

Chart 4.—Composite graph of the three series of experiments. The straight line represents the normal rate of disappearance of edema. The interrupted line shows the disappearance of edema in animals treated with salyrgan. The interrupted line with short and long dots illustrates the diminution of edema following exposure to roentgen ray. The diminution of edema is calculated as a percentual decrease from the height of edema, the maximum increase of each series being 100 per cent. The curve for the x-ray animals coincides very closely with the curve of the animals treated with salyrgan. Both of those curves dip more rapidly than the control curve.

curred immediately following the x-ray exposure. This was occasionally followed by a slight secondary increase. In these animals, as in the others mentioned, no attempt was made to limit the fluid and salt intake. In Chart 4 are

seen the superimposed disappearance curves of all three groups, shown in percentual decrease, rather than in units of measure. The curve for the x-ray animals coincides very closely with that for the salyrgan treated ones. Both of these revealed more rapid disappearance than in the controls.

DISCUSSION

An early disappearance or diminution of the acute thrombophlebitic edema is obviously desirable, if permanent tissue changes are to be prevented or minimized. The response of such an edema to salyrgan, a mercurial diuretic, offers additional evidence of the extrarenal action of this drug. Its clinical use, combined with maximal elevation of the limb and restriction of fluid and salt intake, and followed by gradual exercise and massage, has given gratifying results.[2] The attempt to diminish pain and edema of the limb with x-ray has a purely empirical basis, and our preliminary results would indicate that further work on optimal dosage, filtration and time interval between treatments, is justifiable. The mechanism of its action is obscure, and one can only speculate as to whether it exerts an influence upon the lymphatic flow, or causes a breaking down of lymphocytes and leucocytes with the liberation of proteolytic enzymes and digestion of the protein-rich exudate to a more absorbable form, or results in an ionization of the tissue fluids, or systemically affects fluid mobilization. A more exact knowledge of the effects of roentgen radiation upon normal tissues must be available[4] before an analysis of effects in pathologic conditions can be undertaken. Possibly several of the above mentioned factors are involved in the more rapid disappearance of an acute lymphangitis or periphlebitis such as we have been able to observe clinically.[5] One of us has observed beneficial effects in the treatment of latent infections in varicose veins by means of x-ray.[6] Further clinical and experimental studies are under way to determine the value, limitations and possible dangers of x-ray treatment in acute thrombophlebitis.

SUMMARY

Acute thrombophlebitic edema has been produced in dogs by the injection of a concentrated tissue extract into the femoral vein, and the normal rate of disappearance of the fluid has been determined in a control series of twenty animals. In a second series of twenty dogs, edema was produced in the same manner. After the edema had reached its height, salyrgan, in doses of 1 c.c., was injected intravenously. A rapid diminution of the edema followed, which definitely exceeded the normal rate of disappearance. In a third series of twelve dogs, the edematous limbs were treated with x-ray. The curve of disappearance of the edema in this group was similar to that in the salyrgan treated animals. A possible interpretation and the clinical application of these findings has been briefly discussed.

We wish to express our sincere gratitude to Dr. Earl R. Crowder of the Department of Radiology, Northwestern University Medical School, who gave the roentgen treatments and offered many valuable suggestions during the course of experiments.

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A COMPARATIVE STUDY OF CERTAIN XANTHINE DIURETICS*

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INTRODUCTION

CAFFEINE, theophylline, and theobromine have been made the subjects of extensive studies by many investigators, but no reliable quantitative clinical experiments concerning the comparative diuretic effects of the various xanthine derivatives in graduated doses have been reported. With the exception of one quantitative report in German,[1] there are no published reports of experimental work on laboratory animals with the newer theobromine and theophylline derivatives administered in graduated doses. The conflicting statements in the literature, and numerous inquiries concerning the diuretic powers of the newer derivatives of the methylxanthines prompted these investigations of theophylline calcium salicylate (phyllicin), theobromine calcium salicylate (theocalcin), theophylline sodium acetate (theocin soluble), theobromine sodium salicylate (diuretin), theophylline, and theobromine.

When the studies were begun, the primary objects were to determine what influences these agents, in graduated doses, have on diuresis, and to establish relatively safe maximum doses, so far as determinable effects on the gastrointestinal tract and on the kidneys are concerned. During the course of the work several additional factors presented themselves, the chief one being the influence exerted by these agents on the excretion of uric acid. However, during these studies no attempts were made to discover how these drugs actually increase the uric acid content of the urine, or by what mechanism they influence diuresis,[2] controversial points in the literature.

The consensus of opinion appears to be that, although xanthine possesses little diuretic power, certain derivatives containing the xanthine nucleus are among the most powerful diuretic drugs. Underhill and Pack[3] state that caffeine, theobromine, and theophylline are without doubt the peers of drug

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diuretics for man, and that rabbits likewise are quite responsive to the diuretic influences of these compounds. These investigators found that intravenous injections of the foregoing xanthine derivatives do not increase the volume of urine output. Of the dimethylxanthines, theophylline and theobromine are the more active, while caffeine is the most effective of the trimethylxanthines. Some investigators consider theobromine to be more reliable and less prone to produce disagreeable nervous reactions, while others claim it is apt to prove irritating to the kidneys. Theophylline is credited by many with being a more prompt diuretic, and its diuretic action is supposedly of shorter duration. However, this drug is said to be more irritating to the kidneys and the gastrointestinal tract.

Caffeine, theophylline, and theobromine may fail to promote diuresis if there is not an excess of water in the tissues, as with rabbits which have been kept on a dry diet for a long period of time. These drugs, apparently, hasten the excretion of excess water.[4] On the other hand it has been found that caffeine may fail to increase diuresis even when water is at the disposal of the animal. Schroeder,[5] in 1886, demonstrated that caffeine invariably acts as a diuretic in rabbits if the nerves supplying the kidneys are first sectioned, or if paraldehyde or chloral hydrate are administered first to the animals, measures thought to prevent an interfering vasomotor stimulation. In the dog, diuresis is always obtained if the kidneys are denervated first or the vasomotors paralyzed, and section of the vagus nerves results in increased diuresis or induces it when absent. These observations have led to the conclusion that the vagus nerves carry inhibitory fibers to the secretory cells of the dog's kidney, and that caffeine stimulates these fibers.[6]

Wallace and Pellini[7] showed that theophylline, theobromine, caffeine, and "diuretin" not only failed to produce a diuretic action on dogs which had been kept on a fixed food and water intake, but actually demonstrated a decided decrease in the daily output of urine which often amounted to 50 per cent of the normal volume. They found also that the sodium chloride content was diminished. These workers suggested that there is a shift of water and sodium chloride from the circulating blood to the tissues, and that caffeine and its allies bring this about through the production of a dilatation and an increased permeability of the capillaries in the splanchnic organs and muscles, these effects overshadowing the kidney action in animals which are in water balance. The diuretic effects, then, appear when there is an excess of water in the body, or when the tissues give off some of their stored water. Widmer[8] claimed that the efficiency of caffeine as a diuretic is proportional to the amount of body fluid.

Most writers state that the effects of theobromine on the kidneys are practically identical with those of caffeine. Solis-Cohen and Githens[9] claim that theobromine differs from caffeine in not stimulating the vasomotor and the inhibitosecretory nerves of the kidneys, and that it consequently produces diuresis constantly in dogs and in rabbits. Sasaki[10] demonstrated that the continued administration of theobromine increased water excretion in rabbits only when these animals were kept on a wet diet; but that chloride excretion is increased

also on a dry diet, i.e., independently of a diuretic action. Hellin and Spiro[11] found that caffeine is effective in pure tubular nephritis, but that it fails in glomerular and general nephritis. They reported that theophylline and theobromine are more effective. Pearce, Hill and Eisenbrey,[12] and Schlayer and Hedinger[13] confirmed these observations. Many consider these findings as important evidence that the diuretic effect of caffeine is exerted on the glomerular circulation and filtration, and not on the tubular epithelium. Solis-Cohen and Githens[14] state that when albumin is present, the total amount is generally not increased by the ingestion of caffeine, so that the percentage is diminished because of the increased output of water. These writers state further that occasionally the total amount of albumin may be increased. Sollmann[15] states that caffeine may increase the albumin in nephritis. According to Vinci[16] large doses of caffeine (40 mg. per kilo), long continued especially, produce definite nephritic changes; that dogs are more susceptible than rabbits; but that no lesions have been observed following doses of from 5 to 10 mg. per kilo in man.

Sollmann and McComb[17] state that caffeine in ordinary doses does not appear to produce any demonstrable injury even in nephritic subjects, but that in larger doses it may increase albuminuria. They condemn the continuous use of theophylline even in moderate doses. Emerson[18] reported no increase in albumin in acute or chronic nephritis following the administration of theobromine. Miller[19] is of the opinion that theophylline and theobromine may cause irritation of the stomach and kidneys. Pouchét and Chevalier[20] claim that theophylline in large doses injures both the glomerular and the tubular epithelia. Chevalier[21] reported the presence of toxic impurities in commercial samples of theobromine. Vieth and Leube[1] determined the most effective daily doses of theobromine, "diuretin," "theocalcin," "phyllicin," etc., in rabbits. They concluded that of the acid groups introduced in position one of the theobromine molecule, only the acetyl group increased diuresis. Bürgi's[22] assumption that the diuretic powers of the xanthine derivatives are enhanced by salts was confirmed by these investigators in their experimental work with certain salicylates. They demonstrated that the calcium salts of the xanthine derivatives may influence diuresis in rabbits if this metal is combined with salicylic acid.

Stewart[23] observed that, in man, theobromine calcium salicylate produced less gastric irritation than theophylline and theobromine sodium salicylates; and that this drug may be administered daily for months without untoward symptoms. Selig[24] is one of the very few writers who has reported the effect of theobromine calcium salicylate on the volume of urine excreted by man. He observed that this drug induces diuresis. Cushny[25] states that, when large doses of theobromine are taken for some time, it is prone to act on the stomach and cause loss of appetite and nausea; and that theophylline in a number of cases has had a deleterious action on the stomach, and has caused renal irritation and epileptiform convulsions. He states further that caffeine does not injure the kidney even in large doses and over prolonged periods, and that its administration, therefore, in renal disorders is without risk of increasing the lesions. Solis-Cohen and Githens[26] state that in normal men it has been found that the

average increase in the twenty-four-hour urine resulting from a single dose of 0.5 gm. of caffeine is 42 per cent; and that in rabbits this is true if the doses are below 10 mg.

The literature contains numerous reports of studies concerning the effects of ingested caffeine and other methylated xanthines on the excretion of uric acid, but the reports recorded vary widely. Some investigators state that caffeine increases the uric acid content of the urine; others have found no change in the excretion of uric acid. The weight of published evidence in the earlier literature favors the former view. In 1850 Lehmann[27] reported that no traces of caffeine were found in the urine after the administration of caffeine. The isolation of methylated purines from normal human urine in 1895 was followed by the reports of Albanese[28] that the ingestion of caffeine and of theobromine in dogs resulted in the appearance of monomethyl-xanthine in the urine. During the period 1885 to 1905 Bodzynski and Gottlieb,[29, 30] and Krüger and Schmidt[31, 32] obtained similar results with theobromine and with theophylline when administered to dogs or rabbits. Taylor[33] reported an increase in uric acid following the drinking of coffee. Burian and Schur[34] found that the administration of caffeine failed to produce any effect on the amount of uric acid in the urine. Hess and Schmoll[35] reported an increase in the uric acid after the drinking of coffee and tea. Haig[36] observed an increase in the amount of uric acid following the ingestion of coffee.

In 1916 Benedict[37] reported an increase in uric acid following the administration of caffeine, and Brugsch and Schittenhelm[38] and Mendel and Wardell[39] obtained similar results with caffeine, coffee and tea. These investigators believe that the increased uric acid excretion is possibly due to the demethylation and subsequent oxidation of at least a part of the ingested methylxanthines. The investigations of Mendel and Wardell showed that there is a marked increase in the excretion of uric acid following the ingestion of caffeine, coffee and tea, and that the increase is apparently proportional to the quantity of caffeine ingested. They found that the increase in the amount of uric acid excreted is equal to the quantity of uric acid which would be obtained by the demethylation and subsequent oxidation of from 10 to 15 per cent of the ingested caffeine.

Clark and deLorimer[40] found that caffeine increased the excretion of uric acid in the urine, but that the primary increase was usually followed by a decrease in the excretion; and that theobromine diminished the rate of excretion of uric acid although there was no apparent change in the rate of its formation. These investigators concluded that the increased uric acid excretion following the administration of caffeine is probably due to direct oxidation of the methyxanthines, and suggested that in a general stimulation of metabolism, the kidney cells react first to the increased endogenous uric acid causing an increased excretion, and subsequently the kidney excreting cells react to the foreign and more potent compounds, caffeine or various demethylated derivatives, resulting in a specific excretion of these substances to the partial exclusion of uric acid. Cushny[41] states that the uric acid of the urine is not increased by caffeine, theobromine, or theophylline. Bastedo[42] states that caffeine in

large doses increases metabolism; and that caffeine tends to lose its methyl groups as it passes through the organism, with the resulting formation of monomethylxanthines, xanthine and urea which are excreted in the urine. Means, Aub, and DuBois[43] demonstrated a definite increase in the basal metabolism in man following doses of from 0.5 to 0.7 gm. of caffeine. Myers and Wardell[44] observed an increase in the urinary uric acid after the ingestion of caffeine and theophylline, but not after theobromine. These workers found that when about 300 mg. of theophylline was administered there was an increase of 100 mg. in the uric acid excreted in the urine, a quantity which represents the transformation of 33 per cent of the theophylline ingested. Taylor and Rose[45] confirmed the conclusion of Burian and Schur[46] that part of the nitrogen of ingested purine is excreted at once as uric acid, while part meets an unknown fate. Koehler,[47] and Folin, Berglund and Derick[48] have also confirmed Burian and Schur. Mendel and Lyman[49] found that hypoxanthine ingested by man caused an excretion of 60 per cent of the equivalent amount of uric acid, while xanthine caused 50 per cent, guanine 25 per cent, and adenine 34 per cent. Rose[50] concluded that caffeine and its allies are excreted as such, without the formation of uric acid, and that it was uncertain whether they have any effect on the excretion of uric acid from other sources. Magath[51] claims that the uric acid in the urine is definitely increased after the administration of caffeine.

According to Wiechowski,[52] in dogs, the increased output of uric acid after its parenteral introduction has never been reported higher than 21 per cent. For rabbits, Burian and Schur[46] reported 16 per cent, Wiechowski[52] 5.6 per cent, Bendix and Schittenhelm[53] 18 per cent, and Croftan[54] from 11 to 17 per cent.

Homer[55] found that when dogs are placed on diets which are rich in meat, appreciable amounts of uric acid were eliminated in the urine, 500 gm. of meat per day yields from 0.2 to 0.3 gm. of urinary uric acid in twenty-four hours.

Burian and Schur[56] observed an increase in the hourly excretion of uric acid in dogs which had been given uric acid intravenously, the increase being most marked during the first hour or two. Later there was a fall, and the twenty-four-hour uric acid output was not increased. These investigators explained this on the basis that the rate of the normal destruction of uric acid in the body of the dog was disturbed during diuresis, probably due to circulatory changes; and conversely not due to an increased production of uric acid.

Morris and Rees,[57] who used isotonic and hypertonic solutions of sodium chloride and sodium sulphate with rats and with rabbits, observed that strenuous diuresis increased the total uric acid output in twenty-four hours, and that, as with urea, the output of uric acid is affected by the volume of the urine excreted more than any other factor. They found no evidence of uric acid arising directly from increased kidney activity. Their first series of observations were made with rats and extended over a premedication period of three days and a two-day period following the administration of one dose of the drug, given on the fourth observation day. They found with the sodium sulphate rats an average increase in uric acid output of 59 per cent at the end of the day on which the drug had been administered, as compared with the

average of the three premedication days. The chloride content was increased also. The sodium chloride rats showed a 42 per cent gain in uric acid output. The lowest gain in both groups of animals was 2 per cent, and the highest 66 per cent. The average twenty-four-hour output of uric acid for the sodium sulphate animals was 0.87 mg. per 100 gm. of body weight, and for the sodium chloride animals 0.65 mg. Hourly collections through twenty-four hours made with a second group of rats disclosed the fact that isotonic or hypertonic sodium sulphate solutions cause a large excretion of uric acid in the first or second hour with a compensatory drop later; while the sodium chloride solutions tend toward a more even level in output. The average values obtained for the twenty-four-hour uric acid output were: (1) with sodium sulphate, 0.88 mg. per 100 gm. of body weight, and (2) with sodium chloride, 0.98 mg. If these animals are grouped according to the concentration of the ingested fluids, hypertonic solutions produce 1.01 mg., and isotonic solutions, 0.81 mg. Rats of a third series were given three doses of the drugs at two-hour intervals. The sulphate animals showed a drop in the level of uric acid excreted with each administration, whereas the chloride animals showed a tendency to maintain a more uniform excretion. However, the total six-hour excretion, when calculated on the basis of weight, was practically the same in four out of five animals. There was no significant difference in the twenty-four-hour amount, the sulphate animals giving 1.55 mg., and the chloride animals 1.44 mg. In a fourth series with rabbits observations were made at twenty-minute intervals over four hours, and two drug doses two hours apart were administered. These animals yielded results which were less uniform than the rats, but they were individually consistent. The average maximum yield with the rabbits during the first two-hour period was 13.4 mg. of uric acid per 100 c.c. (the lowest being 6.3, and the highest 18.2), as compared with an average of 8.4 mg. per 100 c.c. obtained at the end of the first twenty-minute period (the lowest, 4; the highest, 11). For the second two-hour period the average maximum yield was 12.7 mg. of uric acid per 100 c.c. (the lowest, 8.5; the highest 15.4).

EXPERIMENTAL

Drugs.—Theophylline calcium salicylate (phyllicin), theobromine calcium salicylate (theocalcin), theophylline sodium acetate (theocin soluble), theobromine sodium salicylate (diuretin), theophylline, and theobromine are the drugs studied in this investigation.

Methods.—The data were from 149 adult rabbits which were kept on a complete and balanced diet determined for the individual animal. The daily water-intake was carefully controlled. The animals were housed in individual metabolic cages, and new animals were used for each dose series. The animals were observed first over a period of days to establish whether they were in a state of metabolic equilibrium. The drugs, dissolved in or mixed with definite volumes of water, were administered by stomach tube three times a day (morning, noon, and evening) over a period of four days. The observations

TABLE I

DIURETIC EFFECTS OF CERTAIN XANTHINES ON RABBITS. UNIFORM FLUID INTAKE: 150 C.C. DAILY. DRUG GIVEN ON FIFTH THROUGH EIGHTH DAYS

ANIMAL	DRUG AND DOSE GM. T.I.D.	DAY	TOTAL DAILY OUTPUT	COLOR TURB. REACT.	S/G	TOTAL SOLIDS GM. P. C.C.	URIC ACID GM. P. L.	UREA MG. P. C.C.	ALBUM. GM. P. L.	SUGAR PER CENT	MICRO-SCOPIC	OTHER ABNOR-MALITIES
7	Normal premedication	1	55	*y/s/al	1.013	0.030	0.17	0.024	0.25	neg.	neg.	neg.
		2	60	y/m/al	1.014	0.033	0.17	0.016	0.25	neg.	neg.	neg.
		3	88	y/m/al	1.015	0.035	0.18	0.014	0.25	neg.	neg.	neg.
		4	41	y/m/al	1.012	0.027	0.19	0.024	0.25	neg.	neg.	neg.
	Averages		61	y/m/al	1.014	0.031	0.18	0.019	0.25	neg.	neg.	neg.
	Phyllicin 20 mg. per kilo.	5	143	a/s/ac	1.014	0.033	0.17	0.018	0.2	neg.	neg.	neg.
		6	70	a/s/al	1.025	0.057	0.25	0.012	tr.	neg.	neg.	neg.
		7	70	y/m/al	1.018	0.042	0.25	0.014	tr.	neg.	neg.	neg.
		8	85	y/m/al	1.014	0.033	0.24	0.014	0.2	neg.	neg.	neg.
	Averages		92	a y/sm/al	1.018	0.041	0.23	0.017	0.1	neg.	neg.	neg.
	Postmedication	9	70	y/s/ac	1.016	0.037	0.17	0.015	0.1	neg.	neg.	neg.
		10	85	y/s/al	1.014	0.033	0.14	0.012	0.3	neg.	neg.	neg.
		11	55	y/m/al	1.012	0.027	0.12	0.012	tr.	neg.	neg.	neg.
		12	59	y/m/al	1.012	0.027	0.12	0.010	0.2	neg.	neg.	neg.
	Averages		67	y/sm/al	1.013	0.030	0.14	0.012	0.15	neg.	neg.	neg.

*y, yellow. s, slight. al, alkaline. a, amber. m, moderate. ac, acid.

were continued thereafter until the daily output of urine approached that of the premedication period. Twenty-four-hour specimens of urine were collected, and the daily observations included the total volume of urine voided, the determination of the physical characteristics of the urine, the specific gravity, reaction, total solids, uric acid, urea, albumin, sugar, and microscopic examination.

Specific gravities were determined by the pyknometer method. Total solids were estimated by the coefficient of Haeser, since the quantities of urine were limited. Uric acid was determined by the method of Morris and Macleod.[58] Urea was estimated by an adaptation of the method of Folin and Wu[59] for blood analyses. Albumin was tested qualitatively by Roberts' test,[60] and, in the event a positive reaction was obtained, Esbach's method[61] was employed for quantitative determinations because of the limited amounts of urine. Sugar was tested qualitatively by Benedict's test.[62] When positive, Benedict's method[63] was employed for quantitative determinations. Chlorides were estimated by Whitehorn's method.[64] Allantoin determinations were attempted by Wiechowski's method,[65] but the results were unsatisfactory because of the small amounts of urine and the dilution. Each specimen was centrifugalized and the sediment was examined microscopically.

Table I shows the type of daily records which were kept for each of the 149 animals used.

OBSERVATIONS

Series I. Theophylline Calcium Salicylate.—This drug, in doses of 10, 20, 40, 50, 60, 75, 100 and 150 mg. per kilo three times a day, was administered to a series of 37 animals. The results obtained (Table II) show that with rabbits 60 mg. doses are the most efficient, if measured by increased urine output and maximum twenty-four-hour output, and that 50 mg. doses are most efficient from the standpoint of increased uric acid elimination and the duration of increased diuresis. There was no evidence of local irritation until doses of 100 mg. or more were reached, when the animals began to manifest loss of ap-

TABLE II

EFFECTS OF VARIOUS DOSES OF PHYLLICIN ON RABBITS

DOSE MG. PER KILO T.I.D.	AVER. % INCR'D OP.	AVER. % INCR'D MAX. OP.	AVER. % INCR'D SOLIDS	AVER. % INCR'D URIC A.	INCR'D ALBUMIN	INCR'D SUGAR	DURATION DIURESIS THROUGH	DAY(S) MAXIM. OP.
10	27	75	18	125	–	–	7½ days	1, 3 dg.;* 2 p.
20	45	93	73	140	–	–	7 days	1, 4 dg.; 1, 2 p.
40	43	81	23	184	–	–	7 days	1, 4 dg.; 2 p.
50	74	140	79	395½	–	–	9 days	1, 2, 3 dg.; 2, 3 p.
60	106	188	86	233	–	–	7½ days	1 dg.
75	94	125	94	280	–	–	8 days	1, 3 dg.
100	105	176	68	537½	plus	–	8 days	1 dg.; 3 p.
150	–34 to +81	---	27	100	Four of seven animals died on the first or second drug day. Casts in urine: gastrointestinal irritation.			

*1, 2, 3 dg. = 1st, 2nd, 3rd drug day throughout tables. 1, 2, 3 p. = 1st, 2nd, 3rd postmedication day. OP. = output.

petite and diarrhea, and increased albumin in the urine. In cases of death, autopsy disclosed gastroenteritis and renal irritation. No deaths occurred until doses of 150 mg. or more were administered.

Series II. Theobromine Calcium Salicylate.—Doses of 10, 30, 50, 75, 100, 150, and 400 mg. per kilo three times a day were administered to a series of 27 rabbits. In the case of this drug (Table III) 50 mg. doses appear to be the most

TABLE III

EFFECTS OF VARIOUS DOSES OF THEOCALCIN ON RABBITS

DOSE MG. PER KILO T.I.D.	AVER. % INCR'D OP.	AVER. % INCR'D MAX. OP.	AVER. % INCR'D SOLIDS	AVER. % INCR'D URIC A.	INCR'D ALBUMIN	INCR'D SUGAR	DURATION DIURESIS THROUGH	DAY(S) MAXIM. OP.
10	61½	110	67	217½	–	–	7½ days	1, 2, 3 dg.
30	22	78½	44	180	–	–	6½ days	1 dg.; 2 p.
50	97	210	48½	187½	–	–	8½ days	1 dg.
75	30	82	19	134	–	–	7 days	1, 2, 3 dg.
100	58½	136	56½	227	plus	–	7 days	1 dg.
150	−14 to plus 50	---	54	131	–	plus	-------	----------
					One animal died second drug day			
400	−10 to plus 179½	---	39	39	---	plus	-------	----------
					All animals died second drug day			

Urinary casts and gastrointestinal irritation were observed in all animals that died

efficient. Although 100 mg. doses produced the greatest increase in uric acid eliminated, irritation resulted from such quantities, and deaths occurred when doses of 150 mg. and over were given.

Series III. Theophylline Sodium Acetate.—Twenty animals were used with this drug which was administered in doses ranging from 10 mg. to 100 mg. per kilo three times a day. Thirty milligram doses (Table IV) produced the

TABLE IV

EFFECTS OF VARIOUS DOSES OF THEOCIN SOLUBLE ON RABBITS

DOSE MG. PER KILO T.I.D	AVER. % INCR'D OP.	AVER. % INCR'D MAX. OP.	AVER. % INCR'D SOLIDS	AVER. % INCR'D URIC A.	INCR'D ALBUMIN	INCR'D SUGAR	DURATION DIURESIS THROUGH	DAY(S) MAXIM. OP.
10	33	72½	48	154½	–	–	6½ days	1, 3 dg.; 1, 2 p.
30	49	88	58½	189	–	–	6½ days	1, 3 dg.; 2 p.
50	35	96	34	164	plus	–	5 days	1, 3 dg.
75	64	120	36	260	plus	–	7¼ days	1, 3 dg.; 3, 4 p.
	One animal died third drug day							
	Two animals lost weight during drug period							
100	131	100	90	267	plus	–	6½ days	1 dg.; 2 p.
	Two animals died during second drug day							
	All animals that died showed casts in urine, and gastroenteritis							

most efficient results within safe limits. Evidence of irritation was obtained with doses of 50 mg. and over. No deaths occurred until doses of 75 mg. or more were given. In cases of death, autopsy disclosed evidence of irritation of the kidneys and the gastrointestinal tract.

Series IV. Theobromine Sodium Salicylate.—Doses of 10, 30, 50, 75, and 100 mg. per kilo were used with 20 animals. The most efficient dose (Table V) was 50 mg., if determined by the increased urine output and the increase in solids eliminated. Larger doses produced a greater elimination of uric acid,

TABLE V

EFFECTS OF VARIOUS DOSES OF DIURETIN ON RABBITS

DOSE MG. PER KILO T.I.D.	AVER. % INCR'D OP.	AVER. % INCR'D MAX. OP.	AVER. % INCR'D SOLIDS	AVER. % INCR'D URIC A.	INCR'D ALBUMIN	INCR'D SUGAR	DURATION DIURESIS THROUGH	DAY(S) MAXIM. OP.
10	50	92	45	72	–	–	7 days	3, 4 dg.; 1, 6 p.
30	44	94	51	88½	–	–	8 days	1, 4 dg.; 2, 4 p.
50	57	97	52½	158	–	–	7½ days	3, 4 dg.; 1 p.
75	26	108	34½	215	–	–	7¾ days	1 dg.; 2, 3 p.
100	46	115	53	262	–	–	8¼ days	1 dg.; 3, 4 p.

but the increases in the volume of daily urine-output and of solids eliminated were less than those resulting from the 50 mg. doses. The amount of uric acid eliminated increased with the dose. There was no evidence of irritation, and no deaths occurred (maximum dose used was 100 mg.).

Series V. Theophylline.—This drug was administered to 19 animals in doses ranging from 10 mg. to 75 mg. per kilo three times a day. The most efficient dose (Table VI) appears to be 50 mg. However, the results obtained with doses

TABLE VI

EFFECTS OF VARIOUS DOSES OF THEOPHYLLINE ON RABBITS

DOSE MG. PER KILO T.I.D.	AVER. % INCR'D OP.	AVER. % INCR'D MAX. OP.	AVER. % INCR'D SOLIDS	AVER. % INCR'D URIC A.	INCR'D ALBUMIN	INCR'D SUGAR	DURATION DIURESIS THROUGH	DAY(S) MAXIM. OP.
10	53	146	37½	72	–	–	9½ days	2, 3 dg.; 2, 3 p.
20	36	78	33	117	–	–	6 days	1, 3 dg.; 2 p.
30	53½	118	18	152	–	–	7⅔ days	1, 3 dg.; 3 p.
50	99½	162	69	222½	–	–	7⅔ days	1, 2, 3 dg.; 3 p.
75	51	174	56½	192				
					Three out of four animals died; one on first postmedication day; the others on the second drug day. Casts in urine, and gastroenteritis.			

TABLE VII

EFFECTS OF VARIOUS DOSES OF THEOBROMINE ON RABBITS

DOSE MG. PER KILO T.I.D.	AVER. % INCR'D OP.	AVER. % INCR'D MAX. OP.	AVER. % INCR'D SOLIDS	AVER. % INCR'D URIC A.	INCR'D ALBUMIN	INCR'D SUGAR	DURATION DIURESIS THROUGH	DAY(S) MAXIM. OP.
10	68	123	19	92½	–	–	8½ days	2, 3 dg.; 3, 4 p.
20	72	130	32	130	–	–	8½ days	1, 3, 4 dg.; 1, 2, 4 p.
40	55	130	65	162½	–	–	9⅓ days	1, 4 dg.; 1, 4 p.
60	28½	95	26	171	–	–	7¾ days	1, 3 dg.; 2, 3 p.
75	43	73	25	199	–	–	8⅔ days	2, 3, 4 dg.; 3, 4 p.
					One animal died during second drug night			
100	65	189½	31	135				
					Three out of four animals died; two during first drug night, one during fourth drug night			
200	97	203	134	208				
					All animals died during third or fourth drug night.			
					Casts in urine, and gastroenteritis in all animals that died			

of 10 mg. as compared with those resulting from the administration of 20, 30, and 75 mg. doses are interesting. The maximum dose (75 mg.) produced death in 3 out of 4 animals, the kidneys and the gastrointestinal tract showing the effects of irritation.

Series VI. Theobromine.—Graduated doses of this drug ranging from 10 mg. to 200 mg. were given to 26 animals. The most effective safe dose from the standpoints of increased maximum output of urine, increased solids, increased elimination of uric acid, and duration of diuresis, is 40 mg. (Table VII), although 20 mg. doses produced a greater increased daily urine output. Deaths occurred with doses of 75 mg. and above, and autopsy showed renal and gastrointestinal irritation in each fatal case.

DISCUSSION

From a consideration of the foregoing data it is evident that the xanthine derivatives included in this study are capable of producing a good diuresis in rabbits, inducing increased elimination of solids and of uric acid, and maintaining a diuretic action for from two to about five and a half days after the administration of the drug is stopped. It is obvious also that in the overdosage established during this study these agents are capable of producing gastroenteritis, nephritis, and subsequent death, but that there is a fair margin of safety. The increase observed in solids was due primarily to chlorides. In general, within safe limits, it appears that when the urine output is increased, the rate of chloride excretion is accelerated until an optimum plateau is reached. Veil and Spiro[66] found that theophylline causes a prolonged fall of serum chloride even in animals whose kidneys have been removed. They conclude that theophylline affects not only the kidneys but also other parts of the vasculature, promoting the passage of chlorides from the blood into the urine and tissues.

Urine Output.—From the standpoint of increase in daily urine output theobromine calcium salicylate (61½) and theobromine (68 per cent) produced the greater increases in dosage of 10 mg. (see Tables VIII to XII). Theophylline calcium salicylate (45 per cent) in dosage of 20 mg. produced a greater increase in diuresis than 30 mg. doses of theobromine calcium salicylate (22 per cent) and theobromine sodium salicylate (44 per cent), and 20 mg. doses of theophylline (36 per cent); although theobromine (72 per cent) in 20 mg. dosage, and theophylline sodium acetate (49 per cent), and theophylline (53½

TABLE VIII

EFFECTS OF 10 MG. DOSES ON RABBITS

DRUG	AVER. % INCR'D OP.	AVER. % INCR'D MAX. OP.	AVER. % INCR'D SOLIDS	AVER. % INCR'D URIC A.	INCR'D ALBUMIN	INCR'D SUGAR	DURATION DIURESIS THROUGH	DAY(S) MAXIM. OP.
Phyllicin	27	75	18	125	–	–	7½ days	1, 3 dg.; 2 p.
Theocalcin	61½	110	67	217½	–	–	7½ days	1, 2, 3 dg.
Theocin Sol.	33	72½	48	154½	–	–	6½ days	1, 3 dg.; 1, 2 p.
Diuretin	50	92	45	72	–	–	7 days	3, 4 dg.; 1, 6 p.
Theophylline	53	146	37½	72	–	–	9½ days	2, 3 dg.; 2, 3 p.
Theobromine	68	123	19	92½	–	–	8½ days	2, 3 dg.; 3, 4 p.

TABLE IX

EFFECTS OF 20 AND 30 MG. DOSES ON RABBITS

DRUG	AVER. % INCR'D OP.	AVER. % INCR'D MAX. OP.	AVER. % INCR'D SOLIDS	AVER. % INCR'D URIC A.	INCR'D ALBUMIN	INCR'D SUGAR	DURATION DIURESIS THROUGH	DAY(S) MAXIM. OP.
Phyllicin (20)	45	93	73	140	–	–	7 days	1, 4 dg.; 1, 2 p.
Theocalcin (30)	22	78½	44	180	–	–	6½ days	1 dg.; 2 p.
Theocin S (30)	49	88	58½	189	–	–	6½ days	1, 3 dg.; 2 p.
Diuretin (30)	44	94	51	88½	–	–	8 days	1, 4 dg.; 2, 4 p.
Theophylline (20)	36	78	33	117	–	–	6 days	1, 3 dg.; 2 p.
Theophylline (30)	53½	118	18	152	–	–	7⅔ days	1, 3 dg.; 3 p.
Theobromine (20)	72	130	32	130	–	–	8½ days	1, 3, 4 dg.; 1, 2, 4 p.

TABLE X

EFFECTS OF 40 AND 50 MG. DOSES ON RABBITS

DRUG	AVER. % INCR'D OP.	AVER. % INCR'D MAX. OP.	AVER. % INCR'D SOLIDS	AVER. % INCR'D URIC A.	INCR'D ALBUMIN	INCR'D SUGAR	DURATION DIURESIS THROUGH	DAY(S) MAXIM. OP.
Phyllicin (40)	43	81	23	184	–	–	7 days	1, 4 dg.; 2 p.
Phyllicin (50)	74	140	79	395½	–	–	9 days	1, 2, 3 dg.; 2, 3 p.
Theocalcin (50)	97	210	48½	187½	–	–	8½ days	1 dg.
Theocin S (50)	35	96	34	164	plus	–	5 days	1, 3 dg.
Diuretin (50)	57	97	52½	158	–	–	7½ days	3, 4 dg.; 1 p.
Theophylline (50)	99½	162	69	222½	–	–	7⅔ days	1, 2, 3 dg.; 3 p.
Theobromine (40)	55	130	65	162½	–	–	9⅓ days	1, 4 dg.; 1, 4 p.

TABLE XI

EFFECTS OF 60 AND 75 MG. DOSES ON RABBITS

DRUG	AVER. % INCR'D OP.	AVER. % INCR'D MAX. OP.	AVER. % INCR'D SOLIDS	AVER. % INCR'D URIC A.	INCR'D ALBUMIN	INCR'D SUGAR	DURATION DIURESIS THROUGH	DAY(S) MAXIM. OP.
Phyllicin (60)	106	188	86	233	–	–	7½ days	1 dg.
Phyllicin (75)	94	125	94	280	–	–	8 days	1, 3 dg.
Theocalcin (75)	30	82	19	134	–	–	7 days	1, 2, 3 dg.
Theocin S (75)	64	120	36	260	plus	–	7¼ days	1, 3 dg.; 3, 4 p.
	One animal died third drug day. Two lost weight during drug period.							
Diuretin (75)	26	108	34½	215	–	–	7¾ days	1 dg.; 2, 3 p.
Theophylline (75)	51	174	56½	192	Three animals out of four died.			
Theobromine (60)	28½	95	26	171	–	–	7¾ days	1, 3 dg.; 2, 3 p.
Theobromine (75)	43	73	25	199	–	–	8⅔ days	2, 3, 4 dg.; 3, 4 p.
	One animal died second drug day.							

per cent) in 30 mg. doses produced a greater diuretic action. In doses of 50 mg. theophylline (99½ per cent), theobromine calcium salicylate (97 per cent), and theophylline calcium salicylate (74 per cent) topped the list. However, the most striking results were observed with theophylline calcium salicylate in doses of 60 mg. (106 per cent), and of 75 mg. (94 per cent), the increases being from 1.7 to 3.7 times those obtained with 75 mg. doses of the

TABLE XII

EFFECTS OF 100, 150, 200 AND 400 MG. DOSES ON RABBITS

DRUG	AVER. % INCR'D OP.	AVER. % INCR'D MAX. OP	AVER. % INCR'D SOLIDS	AVER. % INCR'D URIC A.	INCR'D ALBUMIN	INCR'D SUGAR	DURATION DIURESIS THROUGH	DAY(S) MAXIM. OP.
Phyllicin (100)	105	176	68	537½	plus	–	8 days	1 dg.; 3 p.
Phyllicin (150)	–34 to +81	– – –	27	100	Four of seven animals died.			
Theocalcin (100)	58½	136	56½	227	plus	–	7 days	1 dg.
Theocalcin (150)	–14 to +50	– – –	54	131	–	plus	One animal died second drug day.	
Theocalcin (400)	–10 to +179½	– – –	39	39	– – –	plus	All animals died second drug day.	
Theocin S (100)	131	100	90	267	plus	–	6½ days	1 dg.; 2 p.
	Two animals died second drug day.							
Diuretin (100)	46	115	53	262	–	–	8¼ days	1 dg.; 3, 4 p.
Theobromine (100)	65	189½	31	135				
	Three out of four animals died during drug period.							
Theobromine (200)	97	203	134	208	All animals died third or fourth drug days.			

other five drugs, theobromine sodium salicylate (26 per cent) having produced the smallest increase in this dosage. Theophylline calcium salicylate (105 per cent) in doses of 100 mg. produced an increased urine output of 105 per cent as compared with 131 per cent in the case of theophylline sodium acetate, 65 per cent in the case of theobromine, 58½ per cent in the case of theobromine calcium salicylate, and 46 per cent in the case of theobromine sodium salicylate. However, deaths occurred in the cases of theophylline sodium acetate and theobromine; theobromine sodium salicylate alone in this dosage gave no indications of renal irritation. The greater efficacy of the calcium derivatives suggests the possibility that the calcium ion enhances the diuretic properties of the xanthine with which it is associated.

Uric Acid.—As shown in the introduction, the effect of the methylated xanthines on uric acid excretion has long been a subject of controversy. The data obtained in this study make it obvious that there is a definite increase in the uric acid excreted. The administration of uric acid or nucleins is followed by an increased excretion of uric acid which is more or less closely proportional to the amount of the agent administered. Since it has been established that demethylation does occur in the body, it seems not unlikely that a part of the ingested methylated xanthines may be completely demethylated, and that resulting products may be subsequently oxidized to uric acid. Thus Burian and Schur[67] found that the uric acid injected subcutaneously into man was excreted in the urine to the extent of approximately 50 per cent, these investigators believing that the other half was broken down into unidentified products. Likewise, when hypoxanthine was ingested, about 50 per cent of its nitrogen was accounted for in the immediate increase of uric acid excreted. Although the amount of uric acid eliminated, following the ingestion of these agents, increases in a roughly uniform manner with the increase in the dose of the drug, the calcium derivatives, theophylline calcium salicylate and theobromine calcium salicylate, are exceptions in that with these agents the doses

of greater diuretic efficacy (within safe limits), as measured by the increase in urine output, produce greater uric acid excretion than larger safe doses. The action of these derivatives is apparently specifically confined to uric acid and does not involve the other nonprotein nitrogenous constituents. It is not easy to explain the mechanism by which some diuretics increase the excretion of one constituent more than that of another. The effect may be on the force with which individual substances are held in the blood and tissues, or it may be a direct effect on the tubule epithelium influencing reabsorption to a different extent.

The percentages in the tables expressing increase in the uric acid excreted may be unintentionally self-exaggerating because of the small amounts of uric acid in the urine before medication. To illustrate: In the case of one rabbit which received 20 mg. doses of theophylline calcium salicylate, the daily output of uric acid during the premedication period averaged 11.7 mg. (or 5.85 mg. per kilo of body weight), and during the medication period the average daily output rose to 31.6 mg. (or 15.8 mg. per kilo of body weight), an increase of 19.9 mg. (or 9.9 mg. per kilo of body weight). This represents an increase of 170 per cent in uric acid, although the increase was but 9.9 mg. per kilo or 0.99 mg. per 100 gm. of body weight.

In 10 mg. doses theobromine calcium salicylate (217½ per cent), theophylline sodium acetate (154 per cent), and theophylline calcium salicylate (125 per cent) produced the greater increases in uric acid eliminated. In 20 mg. doses theophylline calcium salicylate (140 per cent) induced a greater increase than theobromine (130 per cent), and theophylline (117 per cent). In doses of 30 mg. theophylline sodium acetate (189 per cent), theobromine calcium salicylate (180 per cent), theophylline (152 per cent), and theophylline calcium salicylate (140 per cent) were more effective. In 40 mg. dosage theophylline calcium salicylate produced an increase of 184 per cent and theobromine 162½ per cent; while in doses of 50 mg. theophylline calcium salicylate (395 per cent) manifested more than twice the powers of the remainder of the group with the exception of theophylline itself (222½ per cent). In this dosage theobromine sodium salicylate produced the smallest increase (158 per cent) in uric acid eliminated.

In 60 mg. doses theophylline calcium salicylate (233 per cent) effected a greater increase in uric acid elimination than theobromine (151 per cent); and in doses of 75 mg. theophylline calcium salicylate (280 per cent) again topped the list.

When the limits of safety of this group of agents as a whole were reached (doses of 100 mg. and over), theophylline calcium salicylate (537½ per cent) still exceeded all other members of the group in increasing uric acid elimination, although theobromine sodium salicylate (262 per cent) was the only member of the group which in 100 mg. doses manifested no symptoms of poisoning. However, the administration of theobromine (135 per cent) itself in dosage of 100 mg. produced death in three out of four animals.

Duration of Diuretic Action.—In 10 mg. doses theophylline manifested activity over a period of nine and one-half days; theobromine, eight and one-half

days; theophylline calcium salicylate, and theobromine calcium salicylate, seven and one-half days; theophylline sodium acetate, six and one-half days; and theobromine sodium salicylate, six days. In doses of 20 mg. and 30 mg. theobromine (eight and one-half days), theobromine sodium salicylate (eight days), theophylline (seven and two-thirds days), and theophylline calcium salicylate (seven days) produced the more prolonged action.

When doses of 40 mg. and 50 mg. were ingested, theobromine (nine and one-third days), theophylline calcium salicylate (nine days), and theobromine calcium salicylate (eight and one-half days) were the more effective members of the group.

In 60 mg. and in 75 mg. doses theophylline calcium salicylate (seven and one-half and eight and two-third days) and theobromine (seven and three-fourths and eight days) were approximately equally effective, although with the larger dosage one theobromine animal died on the second drug day.

All members of the group are prompt in action, increased diuresis starting in practically all cases on the first drug day.

Toxicity.—Theobromine sodium salicylate is the only member of the group which manifested, in the doses employed, no toxicity. However, it should be noted that in this study this drug was not administered in doses larger than 100 mg., and that theobromine itself in doses of 75 mg. produced death in one out of four animals, while with doses of 200 mg. the mortality was 100 per cent by the third or fourth drug days.

The limit of safety is reached apparently with theobromine, theobromine calcium salicylate, and theophylline when these agents are given in doses of 75 mg. per kilo three times a day. In the cases of theobromine calcium salicylate and theophylline calcium salicylate 150 mg. doses seem to be definitely toxic, although in 100 mg. doses, while showing no fatalities, both drugs produced a definite increase in the albumin in the urine.

Autopsies disclosed positive evidences of both renal and gastrointestinal irritation in all animals that died.

Relative Efficacy.—If the increase in urine output, the increase in solids eliminated, the increase in the elimination of uric acid, safety, and the duration of diuretic action, are taken into consideration (see Table XIII), theophylline calcium salicylate, in doses of from 50 to 60 mg. per kilo three times a day, is most efficacious, and theophylline itself in doses of 50 mg. stands second among the remainder of these xanthine derivatives so far as rabbits are concerned. Theobromine calcium salicylate in doses of 50 mg. stands third, close in efficacy to theophylline.

The results of these studies confirm the statements of Underhill and Pack[3] that theophylline and theobromine are powerful diuretics in rabbits (as well as in man). The consensus of opinion in the literature that (1) theobromine is more reliable than theophylline; (2) that theophylline is a more prompt diuretic but shorter in action and more irritating to the kidneys and the gastrointestinal tract; (3) and that both of these xanthine derivatives are capable of producing irritation of the kidneys and the gastrointestinal tract, agrees only in part with the findings of this investigation. These studies disclosed

TABLE XIII

EFFECTS OF THE MORE EFFICACIOUS DOSES ON RABBITS

DRUG AND MG. PER KILO T.I.D.	AVER. % INCR'D OP.	AVER. % INCR'D MAX. OP.	AVER. % INCR'D SOLIDS	AVER. % INCR'D URIC A.	INCR'D ALBUMIN	INCR'D SUGAR	DURATION DIURESIS THROUGH	DAY(S) MAXIM. OP.
Phyllicin (60)	106	188	86	233	--	--	7½ days	1 dg.
Theophylline (50)	99½	162	69	222½	--	--	7⅔ days	1, 2, 3 dg.; 3 p.
Theocalcin (50)	97	210	48½	187½	--	--	8½ days	1 dg.
Phyllicin (50)	74	140	79	395½	--	--	9 days	1, 2, 3 dg.; 2, 3 p.
Theobromine (20)	72	130	32	130	--	--	8½ days	1, 3, 4 dg.; 1, 2, 4 p.
Diuretin (50)	57	97	52½	158	--	--	7½ days	3, 4 dg.; 1 p.
Theobromine (40)	55	130	65	162½	--	--	9⅓ days	1, 4 dg.; 1, 4 p.
Theocin S (30)	49	88	58½	189	--	--	6½ days	1, 3 dg.; 2 p.

TABLE XIV

TOXIC DOSES OF XANTHINE DIURETICS ON RABBITS

DRUG AND MG. PER KILO T.I.D.	AVER. % INCR'D OP.	AVER. % INCR'D MAX. OP.	AVER. % INCR'D SOLIDS	AVER. % INCR'D URIC A.	*MORTALITY
Theocalcin (150)	−14 to +50	--	54	131	1 out of 4 animals died on 2nd medication day
Phyllicin (150)	−34 to +81	--	27	100	4 out of 7 animals died on 1st or 2nd med. day
Diuretin	No deaths or evidences of renal or gastrointestinal irritation with doses up to and including 100 mg. Larger doses of this drug were not given.				
Theocin S (75)	64	120	36	260	1 out of 4 animals died on 3rd med. day. (NOTE: 3 out of 4 died following 100 mg. doses.)
Theobromine (75)	43	73	25	199	1 out of 4 animals died on 2nd med. day. (NOTE: 3 out of 4 died following 100 mg. doses.)
Theophylline (75)	51	174	56½	192	3 out of 4 animals died; one on 1st post-med. day, two on 2nd med. day.

*Autopsies disclosed definite evidence of renal and gastrointestinal irritation.

the facts that in rabbits (1) theobromine and theophylline and their salts are equally reliable diuretics; (2) that in both of these groups diuresis began promptly within approximately the same time (usually the first medication day), and, in the more efficacious doses, was practically of equal duration; and (3) that renal and gastrointestinal irritation is produced by practically the same dosage of the members of each of these groups (excepting "diuretin," which was not administered in doses larger than 100 mg.).

In clinical doses Stewart[23] found that theobromine calcium salicylate produced less irritation than theobromine sodium salicylate. The findings of this study show that in rabbits with doses up to and including 100 mg. theobromine sodium salicylate gave no indications of irritation, whereas theobromine calcium salicylate in doses of 100 mg. and over produced an increase in urine albumin.

Vieth's and Leube's[1] observation that calcium salts of the xanthines apparently influence diuresis in rabbits is confirmed by the results obtained in these studies.

The conclusion of Emerson[18] that there is no increase in albumin after theobromine is contradicted by these findings, since theobromine in 75 mg. doses and theobromine calcium salicylate in 150 mg. doses both gave definite evidence of an increase in urinary albumin.

The observations of Bodzynski,[29] Gottlieb,[30] Krüger,[31] Schmidt,[32] Hess,[33] Schmoll,[35] Mendel and Wardell,[39] and others that there is an increase in uric acid following the ingestion of theophylline and theobromine are confirmed by this work. The conclusion of Morris and Rees[57] that the output of uric acid is affected by the volume of urine more than any other factor does not agree with the results obtained in these studies.

The relative toxicity of the salts as compared with the free xanthines appears to be about directly proportional to the amount of the xanthine contained therein, with the exception of theophylline sodium acetate which is as toxic as theophylline.

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EMBOLISM AND THROMBOSIS OF LARGE BRANCHES OF THE PULMONARY ARTERY IN HEART DISEASE*

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OBSTRUCTION of the pulmonary artery, like that of other vessels may occur as a result of thrombosis or embolism. The two processes having so much in common, and often so difficult to differentiate are usually considered together in discussions of this subject. Although the literature abounds with general studies on thrombosis and embolism, the relationship between pulmonary obstruction and heart disease has been somewhat neglected.

The study of the mechanism of thrombosis has been vigorously pursued and the definite answer as to how and why intravascular clotting occurs is still to be forthcoming. John Hunter[1] believed that infection of the vessel wall was responsible for thrombosis. Later, Virchow[2] brought forth a noninfectious process as the cause, namely, a sluggish circulation, which with other factors predisposed to the clotting. He applied the term "marantic" to thrombi of such origin. In a study of 1,000 cases of thrombosis and embolism, Rosenthal[3] found the incidence highest in individuals over forty-one years of age in whom heart disease was present. The inference would be that stasis of the blood resulting from a failing myocardium is responsible for thrombus formation. Experimental work, however, has shown that stasis alone, or in association with endothelial injury will not result in thrombus formation. It appears, therefore, that

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changes in the blood itself, physical, chemical and colloidal changes, are necessary in order that thrombus formation take place.

Emboli on the other hand are usually pieces of clot which have broken off. There are in general two types of pulmonary emboli,[4] one in which a large branch of the pulmonary artery is occluded, and the other in which smaller branches are occluded to produce either local disturbances or infarction. A large embolus may pass through the heart and lodge in the trunk of the pulmonary artery.

McCartney[5] in his review of 73 cases of pulmonary embolism has included 16 cases of so-called medical embolism, embolism occurring in nonsurgical cases. Of these 16 cases, 6 were associated with cardiac disease. From this data it may be concluded that about 8 per cent of emboli occur in cardiac disease. All of McCartney's heart cases suffered from hypertensive disease.

In this connection the work of Haggart and Walker[6] is of interest. Studying the effect of quantitative occlusion of the pulmonary artery they found that until from 52 per cent to 66 per cent of the pulmonary circulation is cut off, there is no significant variation in the circulatory condition of the animal. The point at which failure occurs is sharply defined, since beyond this end-point a circulatory collapse is precipitated by minute increases in pulmonary arterial obstruction, but if this is not applied, no untoward change results. This experimental evidence coincides with the clinical findings of Means and Mallory,[7] who report a case of occlusion of the main right branch of the pulmonary artery by an organized thrombus which had been present for a period of many days or even weeks.

The present study is based upon 25 cases of frank cardiac disease, appearing on the wards of Cleveland City Hospital. All cases at autopsy had occlusion of large main branches of the pulmonary arteries. In 6 instances the pathologic diagnosis was that of pulmonary embolus.

Of the 6 cases in which the diagnosis of embolism was made there were 5 males and 1 female. The youngest was thirty-six years of age and the oldest fifty-nine years, the average age being fifty-two years. Of these, 3 cases had mitral stenosis; 1 had hypertensive heart disease; 1 had syphilitic aortitis and general paresis; 1 had coronary disease. Strikingly enough, in all cases the cardiac mechanism was normal. This is of significance, because ordinarily emboli are thought of as occurring with arhythmias, but here none of the cases show such abnormality. In only 1 of these patients was death of the type commonly associated with pulmonary emboli. This case, one of chronic mitral and aortic disease, was found moaning while asleep. Cyanosis and dyspnea developed and death was sudden. In all the other cases death could be attributed to additional factors, the pulmonary embolus being apparently of minor consequence and occurring as a terminal event. For example, 1 case had a cerebral embolus; 1 the wet brain of general paresis; 1 coronary thrombosis; and the other 2 chronic failure of from six to twelve months in duration.

We may conclude from these cases that the typical death attributed to pulmonary embolism, the chest pain, dyspnea, cyanosis and anxiety, may be

often absent when the condition occurs in the course of heart disease. It appears that pulmonary embolism occurs so late in heart disease that it represents a terminal event.

A possible source of the embolus was found in 3 cases. Pulmonary infarcts were present in 2 instances.

In the remaining 19 cases of this study, the pathologic diagnosis was that of thrombosis. The youngest of these was thirty-five years of age and the oldest seventy-three years. There were 8 females and 11 males.

In all but 3 of these, cardiac failure was present to an obvious degree. As near as can be determined, the average time of failure was three months. Thus about 85 per cent of the cases were associated with cardiac failure extending over long periods of time.

The 3 cases which had no failure are of interest. One had coronary disease with myomalacia; another cerebral necrosis with a thrombus straddling the bifurcation of the inferior vena cava; a third had a cerebral hemorrhage.

In the cases then, in which myocardial failure was absent, other conditions were present which were sufficient to produce lethal exitus. In all instances the pulmonary thrombosis may be considered as a condition coming at the termination of an otherwise fatal disease.

In the cases of thrombosis there is again a striking paucity of cardiac arhythmia. Only 5 of the cases, approximately 25 per cent were fibrillating, and there was 1 case of premature beats. As in the process of embolism, auricular fibrillation is not a frequent companion of thrombus formation.

Various types of cardiac disease are represented in this series. Hypertensive heart disease occurred in 13 instances, about 65 per cent. Rheumatic heart disease appeared in 1 case. Syphilitic heart disease appeared 3 times. Coronary disease was present 2 times and chronic pulmonary emphysema once. When studied with respect to the number of these diseases admitted to the hospital, it would seem that hypertensive disease is represented a little more than anticipated and rheumatic heart disease a little less. Embolism is more often associated with rheumatic disease and thrombosis with hypertensive disease.

Pulmonary infarcts were present in 13 instances, 68 per cent of the cases of pulmonary thrombosis, a much higher incidence than in cases of pulmonary emboli.

The frequency of antemortem thrombi in the right side of the heart was studied. In 7 instances there were such thrombi present. In 4 cases the thrombi were in the auricles alone; in 1 case in the right ventricle alone; in 2 cases in both auricles and ventricles.

In 3 additional cases there were thrombi in the venous system. These thrombi were found in the inferior vena cava, the right iliac vein and the gastro-epiploic vein.

The following cases are representative of this series:

L. C., a negro male, forty years of age, was admitted Apr. 19, 1932, and died Aug. 20, 1932. He had been failing for four months. His heart was enlarged, gallop rhythm. Blood pressure was 160/100. Clinical diagnosis: diffuse vascular disease, and congestive cardiac failure. Anatomic diagnosis: nephrosclerosis, cardiac hypertrophy, thrombus right pulmonary artery, thrombosis right auricle and left ventricle, chronic passive congestion, and multiple pulmonary infarcts.

E. N., a negress, thirty-six years of age, was admitted Feb. 21, 1929, and died Mar. 13, 1929. She had had shortness of breath and swelling of ankles for two weeks. On examination she had Cheyne-Stokes respiration, left-sided hemiplegia, enlarged left ventricle, with systolic murmur at apex. Blood pressure was 114/64. Clinical diagnosis: cerebral accident, and either embolism hemorrhage or thrombosis. Anatomic diagnosis: mitral stenosis, mural thrombi, right auricle and ventricle, pulmonary embolus, patent ductus arteriosus, and encephalomalacia,* right.

One of the purposes of a study of this kind is to increase diagnostic skill as a result of the classification of disease. From the diagnostic standpoint there is nothing particularly distinctive about these cases. If, in a case of cardiac disease the typical symptoms of pulmonary embolism appear, the diagnosis is simple. From the cases studied, however, it appears that this is not likely to occur. In heart disease the pulmonary emboli are usually quiescent and give little manifestation of their presence. A similar statement can be made concerning thrombi. It can be said, none the less, that in a case of prolonged cardiac failure, with repeated indication of pulmonary infarction, embolism, or thrombosis of the pulmonary vessels may be anticipated. This is in accord with the opinion expressed by Brenner[8] who in his report of 6 cases of thrombosis of large branches of the pulmonary artery, concludes that thrombosis usually occurs after severe congestive cardiac failure has set in and produces no evident additional symptoms though it probably hastens the end.

CONCLUSIONS

1. Twenty-five cases of cardiac disease with embolism or thrombosis of the larger pulmonary arteries are reviewed.
2. Hypertensive cardiac disease is most frequently associated with pulmonary thrombosis.
3. Rheumatic heart disease is most frequently associated with embolism of the pulmonary arteries.
4. Auricular fibrillation was present in only a few cases.
5. Diagnosis of these conditions usually must be made by inference.

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*This may have been due to a paradoxical embolus passing through the patent ductus arteriosus.

MULTIPLE TUMORS*†

Report of a Case

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WHILE the occurrence of multiple tumors in a single individual is of no definite scientific importance at this time, their presence, nevertheless, arouses interest and never fails to invite speculation as to the reason for their existence.

Puhr, in 1926, published a study of a series of 6,718 necropsies performed during the preceding twenty-one years at the Second Budapest Pathological Institute, finding 1,559 cases of tumors. Two hundred and nineteen of these, or 14.04 per cent of the tumor cases, showed multiple growths. Puhr concludes from his assembled data that the occurrence of multiple tumors in the same individual is common enough to indicate the existence of a "tumor diathesis" either congenital or acquired. It is to be noted that in this series only five of the cases of multiple newgrowths show more than one tumor of the malignant variety.

Egli, quoted by Murray, finds multiplicity of neoplasms in the same individual in 263, or 27.3 per cent, of 966 tumor cases, which were studied during a seven-year period at the Basel Pathological Institute. In twenty of these cases, multiple malignant growths were demonstrated.

Harlitz, quoted by Murray, found 16 cases of multiple malignant growths in 524 cancer autopsies seen during the period of time from 1900 to 1915. These sixteen cases thus represented roughly 3 per cent of the total number.

Murray states that thirty-two examples of multiple malignancy were found in a series of 4,219 examinations made at the Oslo Institute for the Norwegian Cancer Committee. Thus, multiple malignancy was encountered in 1 per cent of the cases.

From a study of the reports of these authors and of various statistical studies concerning the occurrence of malignant tumors, Murray concludes that the multiplicity of neoplasms is rare, particularly the coexistence of tumors of the malignant variety. He feels that there is probably inherent in one malignant tumor a tendency to inhibit the development of other malignant tumors.

Yamakawa has reported a case of coexistent gastric cancer, lung cancer, kidney adenoma and neurofibroma of the suprarenal. Luchsinger reports coexistent cancroid of the forehead, bronchial cancer, malignant hypernephroma and osteochondroma of the lungs. Seecof found coexistent carcinoma

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of the lung and carcinoma of the rectum, each producing typical metastases. Other cases of multiple tumors have been reported by Risak, Siebke, Ball and Reynolds, Perrotti, Jolkwer and others.

We wish to report a case of four different coexistent tumors found at

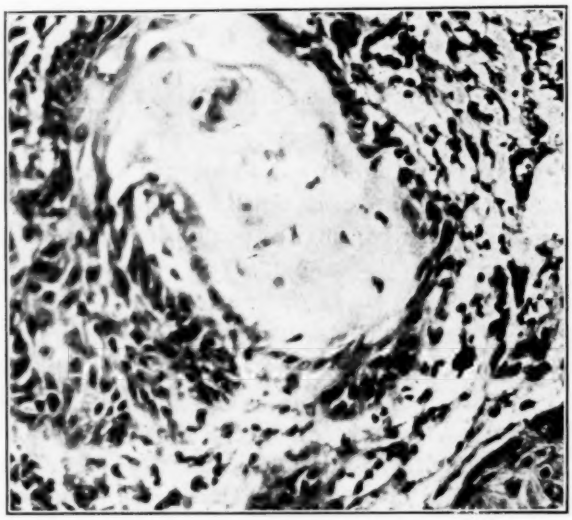

Fig. 1.—Sixteen mm. obj. oc. 15 × camera 50 cm. × 300 reduced. Penile tumor showing formation of epithelial whorl.

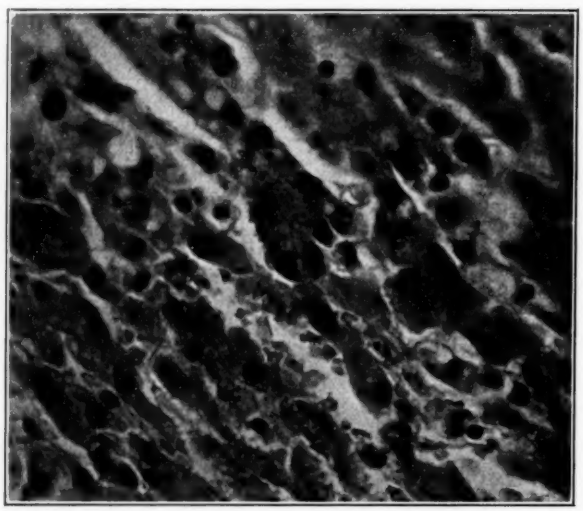

Fig. 2.—Eight mm. obj. oc. 15 × camera 50 cm. × 600 reduced. Hepatic tumor showing gradual transition from liver cords to the ill-defined tumor columns.

autopsy at the Edward Hines Jr. Hospital. This represents the only case showing multiple malignant newgrowths in a series of 364 autopsies.

CASE 1.—J. C., white male, aged sixty-two years, entered the hospital December, 1930, complaining of extreme weakness and loss of weight; a recurrence of penile growth which had occasioned a subtotal amputation about six months previously.

Family History.—Revealed nothing of interest.

Past History.—Patient denied venereal infection. In 1924 a rodent ulcer of the left jaw was removed surgically. There had been no recurrence of this tumor. In October, 1929, a lesion appeared on the foreskin of the penis which was later diagnosed as "cancer" and for which a subtotal amputation of the penis was performed in June, 1930. An ulcer on the

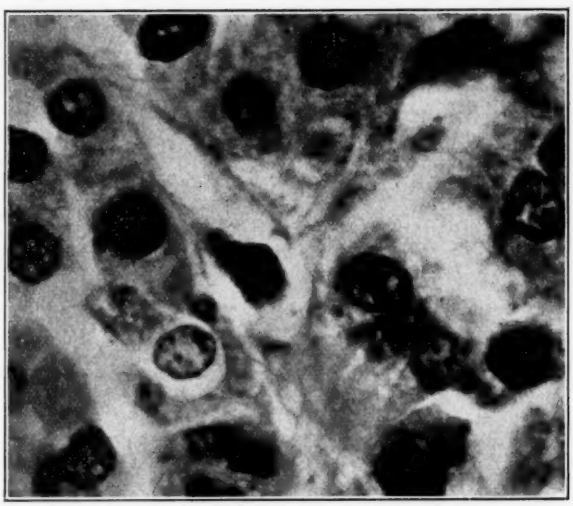

Fig. 3.—Four mm. obj. oc. 15 × camera 50 cm. × 1200 reduced. Liver tumor showing large cloudy cells forming ill-defined cords. Note the mass of pigment within space at center of cut.

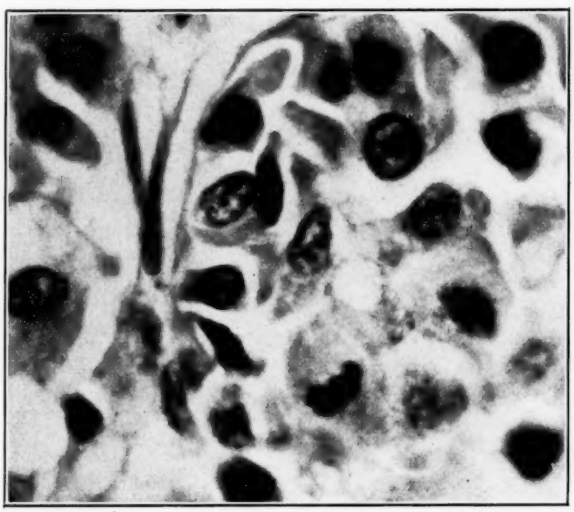

Fig. 4.—Four mm. oc. 15 × camera 50 cm. 1200 × reduced. Liver tumor. Detail showing mitotic figure.

left cheek had been gradually enlarging over a period of ten years until at the present time it is 1.5 cm. in diameter.

Physical examination revealed an emaciated senile male who was in an extreme cachectic state. An ulcer of approximately 1.5 cm. in diameter was seen on the left cheek approximately 2 cm. below the outer canthus of the eye. A large cauliflowerlike growth involved the penoscrotal junction. The nodes of the right inguinal region were enlarged.

The other physical findings were irrelevant.

Clinical Course.—The penile tumor and regional lymph nodes were repeatedly subjected to irradiation. Despite the treatment, the condition of the patient became steadily worse and death resulted on Jan. 7, 1931.

Autopsy resulted in the following anatomic diagnoses: Epidermoid carcinoma penis

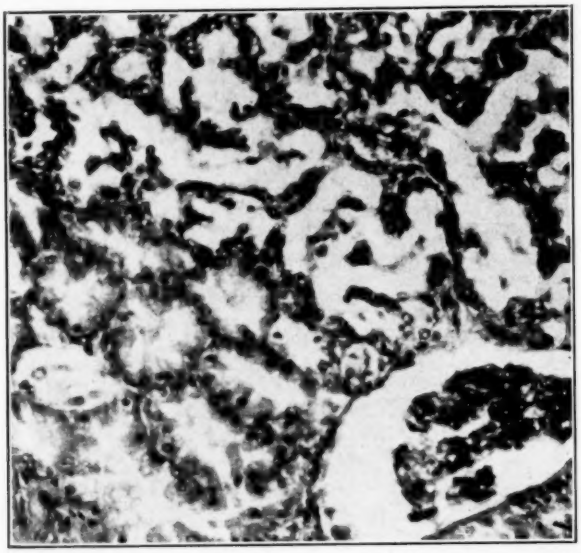

Fig. 5.—Sixteen mm. oc. 15 × camera 50 cm. × 300 reduced. Kidney tumor showing well-defined line of demarcation between renal tissue and atypical tubule formation of neoplasm.

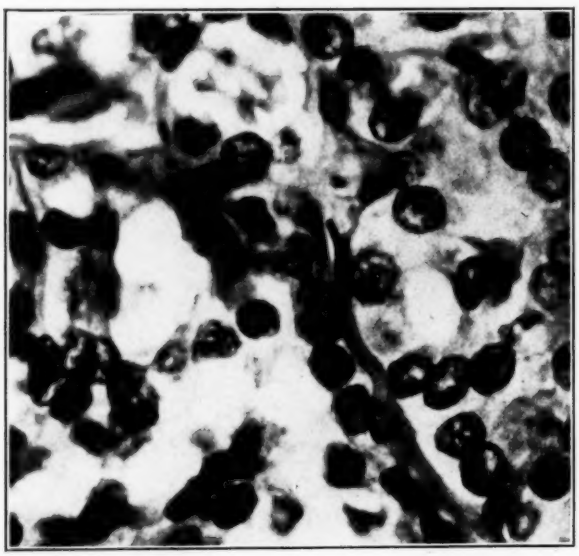

Fig. 6.—Eight mm. oc. 15 × camera 50 cm. × 600 reduced. Details of renal tumor. The atypical tubule formation is noted.

with metastases to regional lymph nodes; adenocarcinoma liver; adenoma kidney, right; carcinoma, basal cell type, face; bronchopneumonia; arteriosclerosis, advanced, severe; nephritis, interstitial, chronic, vascular type; myocardial degeneration; chronic passive congestion of liver; atrophy spleen, senile; hypertrophy prostate, adenomatous; hyperkeratosis, senile, left face; dermatitis, senile, legs.

Description of gross appearance of organs which proved on histologic examination to be the site of distinctly different neoplasms.

1. *Penis.*—The remnant of the penis was destroyed by a fungating cauliflowerlike growth. On section this tumor had a wartlike periphery, the inner portion being pearly white and of a cartilaginous consistency. The glands of both inguinal regions were dense and fibrosed but showed no gross evidence of tumor.

2. *Liver.*—A firm, grayish white nodule 3 cm. in diameter was found on the anterolateral margin of the dome of the right lobe elevating the capsule somewhat. Cross-section through this mass revealed a homogeneous grayish white pearly surface of almost cartilaginous consistency. It was not encapsulated.

3. *Right Kidney.*—This organ weighed 140 gm. A nodule 2 cm. in diameter was located in the middle third of the cortex and bulged beneath the capsule. Section through the nodule revealed a yellowish white, smooth, homogeneous cut surface. This tissue was found to be of a firm consistency, but was not encapsulated although the edges were well defined.

4. *Skin Lesion Noted on Face.*—A shallow ulcer 1 cm. in diameter with elevated borders

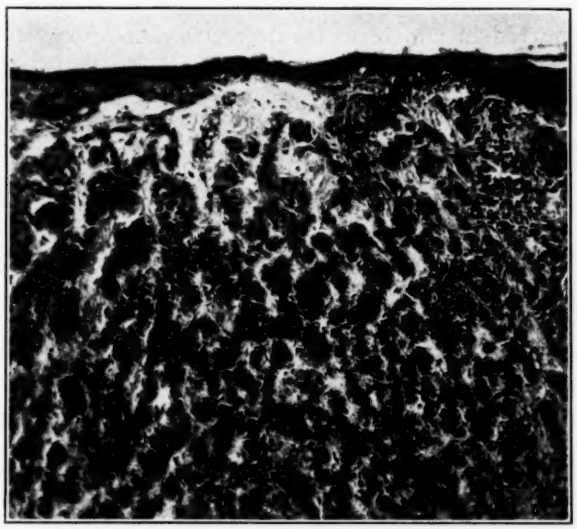

Fig. 7.—Sixteen mm. oc. 15 × camera 50 cm. × 300 reduced. Skin tumor. The epithelial invasion of the lower strata is noted.

of dense consistency was noted over the left molar region. The edge of the ulcer cavity was undermined. The base was filled with recent granulations. This lesion had the gross characteristics of a "rodent ulcer."

Histologic Examination of Penile Tumor.—Examination revealed a proliferation of squamous epithelium which invaded the deeper layers of tissue in long finger-like projections. The cells were well differentiated and contained much keratinization with the formation of epithelial "whorls." Mitotic figures were few in number.

Diagnosis.—Epidermoid carcinoma of penis.

Histologic Examination of Liver Tumor.—A regular epithelial cell proliferation forming ill-defined columns which closely resembled liver cords was noted. The cells were large, some being round and others elongated in shape. These cells showed a cloudy cytoplasm and a large open vesicular nucleus with a well-defined nucleolus. Mitoses were frequent. There was a definite well-marked fibrous stroma which was well vascularized. Indefinite channels, in many of which were small free masses of brown pigment, were formed between the cords of epithelial cells, probably in an ill-formed bile capillary. A gradual transition from the well-differentiated liver cell to the irregular atypical tumor cell existed without definite line of demarcation.

Diagnosis.—Adenocarcinoma of liver.

Histologic Examination of Kidney Tumor.—The epithelial proliferation tended to form columns and well-marked tubules of irregular size on well-vascularized stroma. The cells were large and oval with cloudy reticular cytoplasm. The nuclei were large and hyperchromatic and contained well-defined nucleoli. No mitotic figures were seen. The line of demarcation between the kidney parenchyma and tumor tubules was definite although the tumor was non-encapsulated. The latter lack the uniformity of true renal tubules both as to diameters and cytologic lining.

Diagnosis.—Adenoma of kidney.

Histologic Examination of Skin Lesion.—A well-marked proliferation of the lower layers of the epithelium infiltrated deeply into the submucosa. The cells were small, some round, and some oval with hyperchromatic nuclei. These cells formed masses with pseudocolumnar margins and closely resembled those seen in the basal layer of epidermis. No mitotic figures were noted.

Diagnosis.—Carcinoma, basal cell type.

SUMMARY

A case is reported in which at autopsy was demonstrated the simultaneous occurrence of four tumors, three malignant and one benign. Seven years previously the patient was operated upon for a rodent ulcer which did not recur.

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CHOOSING A SERUM TEST FOR SYPHILIS*†

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IN ADDITION to considerable laboratory experience with several of the numerous modifications of the Wassermann test, and with some of the so-called precipitation or flocculation tests, I have applied these serum tests to the diagnosis and management of syphilis in the clinic. It is, therefore, from a point of view which combines laboratory and clinical experience that I wish to consider the problem of choosing a serum test for syphilis.

The ideal or completely adequate test would be one which would give positive reactions in all cases of syphilis, and in no others. Thus, the first requirement for ideal adequacy would be that the test to be so sensitive as to detect the presence of syphilis from the beginning of the infection to the complete destruction of every *Treponema pallidum* in the body. According to present knowledge, no serum test can be expected to be entirely adequate in regard to this requirement because before the serum can give a positive reaction the *Treponema pallidum* must have resided in the body long enough to have evoked some sort of tissue response which directly or indirectly causes the detectable changes in the serum. Such changes rarely occur sooner than ten days after infection. Furthermore, serum tests are lacking in adequacy, in that, as yet no method has been devised which definitely indicates when the last treponeme has been destroyed. The second essential for an adequate serum test would be that it be specific in all cases. The occurrence of positive reactions with the serum of nonsyphilitic persons is obviously a serious fault.

From a practical standpoint there are certain other essentials for adequacy. The test must be dependable; its technic should be of such a nature that it can be accurately executed by the average laboratory worker, the results being consistent from day to day, and consistent with those obtained in other laboratories performing the same test. Furthermore, in addition to being sensitive, specific, and dependable, a method should be comparatively inexpensive. However accurate a new method is, it will not receive general recognition if the expense of performing the test is much greater than that of some of the excellent methods already in use. Finally, in order to survive, the method must be easy of execution, and easier still of interpretation.

Since it is inconceivable, from present knowledge, that any test could be completely adequate, one should be guided in the selection of a test for syphilis by its helpfulness to the physician.

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COMPLEMENT FIXATION TESTS

Since the Wassermann reaction is the oldest and most widely known test, I shall speak first of its inadequacies:

1. Lack of sensitiveness has taught able clinicians never to disregard unmistakable signs of syphilis merely on the strength of a negative Wassermann reaction. Failures of the Wassermann reaction are approximately 50 per cent in the primary stage of the disease, are few in the secondary stage, and vary between 40 and 60 per cent in the tertiary stage, depending upon treatment, duration, and so-called latency.

2. A positive Wassermann reaction is not always specific for syphilis, because certain acute respiratory infections, particularly pneumonia, and a few other conditions such as malaria and bacterial endocarditis, often cause positive reactions in individuals who are not syphilitic.

3. None of the many modifications of the Wassermann test is entirely dependable. Altogether too frequently there arises a situation in which a positive report is received from one laboratory, and a negative report from another, even though both laboratories are using the same Wassermann technic on separate samples of the same blood. Still more perplexing is such an occurrence as a report of a positive reaction in a given laboratory, followed very shortly, without any clinical reason, by a report of a negative reaction in the same laboratory.

In spite of these imperfections, we must not underestimate the immense contribution of the Wassermann reaction, for in doubling ability to detect the disease, it has, to date, I believe, done more to control syphilis than any other single factor.

FLOCCULATION TESTS

During the last ten years numerous tests have been devised for the purpose of increasing accuracy in the detection of syphilis and of more efficiently excluding it in differential diagnosis. Most of these new tests have made use of the principle, first stated in 1907 by Michaelis, and two years later affirmed by Jacobstahl, that the serum of a syphilitic person, when mixed with a suspension of suitably chosen lipoids, usually produces a change in the dispersion of the lipoids, leading to flocculation; the term "flocculation" is used rather than "precipitation" because flocculation describes a change rather than defines a mechanism, as yet not fully understood. It is not difficult to understand why so much progress has been made in the development of flocculation tests when one remembers that in order to effect a positive reaction with syphilitic serums, only the admixture of lipoids is necessary. On the other hand, the Wassermann reaction or any complement fixation test requires, in addition to the lipoids and the serum to be tested, three different reagents (amboceptor, complement, and sheep's cells), all three of which are biologic products whose respective potencies have never been controllable with certainty. It is now generally admitted that the flocculation tests are more dependable, and in my experience they have proved more specific than any of the complement fixation methods.

As a result of investigations of several flocculation methods in the laboratories under my direction, it has been possible to make the following comparable ratings in terms of adequacy, as expressed by sensitiveness and dependability. In a large group of cases studied it was found that the Eagle test possesses 72 per cent of the adequacy of the Hinton, the Kahn test 70 per cent, the Kline test from 71 to 80 per cent, and the Wassermann test from 38 to 46 per cent. The range of adequacy in the case of the Kline test has depended upon whether the antigen used was prepared in Kline's laboratory or in my own laboratory. With the former I obtained 71 per cent of adequacy, and with the latter I obtained 80 per cent of adequacy, as based on sensitiveness and dependability. With Kline's antigen which I prepared, however, the results suffered considerably in specificity, since there were several nonspecific positive reactions. In the case of the Wassermann reaction, the range was greatly influenced by the type of cases, the sensitiveness being greater among the cases of syphilis from venereal disease clinics than among those of neurosyphilis from psychopathic hospitals.

It thus appears that in regard to sensitiveness and dependability there is little to choose between the Kline, the Kahn, and the Eagle tests. Accordingly, other factors must determine the choice. Each of the tests that I have mentioned presents certain difficulties. The Kahn test is admittedly difficult to read, although an unusual degree of accuracy in reading this test has been attained by those trained in Kahn's laboratory. The Kline test presents very few technical difficulties that are readily discernible; however, in our hands it has had its good and bad days. The difficulty with the Eagle test lies in its technic; in a laboratory in which a large number of tests are done, centrifuging each tube is difficult and impractical. The greatest difficulty with the Hinton test is that despite its simplicity it must be performed with exactness and precision for the best results. This can be obtained only by practice; in the hands of a serologic technician with no experience with the Hinton test, 80 to 90 per cent of adequacy can be obtained in less than a month, while the highest degree of accuracy in testing and reading usually requires experience of several months. Another practical disadvantage is the necessity of overnight incubation. These obstacles, however, I believe are outweighed by the greater sensitiveness and dependability of this test.

I feel strongly on the question of adequate sensitiveness of a test for syphilis; I have seen much suffering and great expense caused by a negative Wassermann reaction which, with absence of clinical signs, caused the physician to conclude that the disease was not present, when actually it existed in a communicable form. Thurmon's[1] figures show that during the first week after the appearance of the primary lesion, the Hinton reaction of the blood was negative in only 38 per cent of the cases studied; that in the second week of the primary lesion only 14 per cent were missed by the Hinton test, and that from the beginning to the end of the third week only 6 per cent of the cases remained negative. It is a well-known fact that in primary syphilis in which more than three weeks have elapsed since the beginning of the initial lesion, serum reactions are practically 100 per cent positive by any worth-while method.

In addition to the value which sensitiveness has for the early detection of syphilis, it has equal, if not greater, importance as an aid in the determination of cure. In the tests with which I have had experience, there has been none except the Hinton test which, in a large group of cases, was always positive whenever the spinal fluid showed a positive reaction by accepted tests.[2] This is of great importance to the general practitioner particularly, for it means that when the Hinton reaction of the blood is negative one will be unable, by lumbar puncture, to disclose laboratory evidence of neurosyphilis.

INTERPRETATION OF STATISTICS

In the determination of the adequacy of a test, statistics, which for the most part must serve as the guide, are often misleading. The two chief reasons for this are: (1) frequently the cases upon which figures are based are insufficiently studied clinically, and (2) the statistics are often erroneously prepared. Any such study, to be valid, must be based upon a complete as well as a careful clinical examination of each case by experts in syphilology. I would, for this reason, exclude all other than institutional cases in a statistical investigation of the adequacy of *any* serum test for syphilis. In referring to erroneously prepared statistics, I have in mind particularly those indicating the percentage of agreement between two methods. I can illustrate a frequently occurring fallacy in statistics by the following: Suppose a laboratory were to test 1,000 nonsyphilitic bloods, using two given methods, one of which was absolutely insensitive. The results would agree unless, perchance, one of the methods gave nonspecific reactions. If, using the same methods, tests are made upon 1,000 individuals, 10 of whom are definitely syphilitic, and 990 of whom are not, and one method finds the bloods of the 10 known syphilitic individuals positive, and the 990 normal persons negative its score will be 100 per cent. The method lacking sensitiveness finds not a single syphilitic's blood positive, though it finds the 990 negative; its true rating would be nil, yet, according to prevailing statistical methods, it would be computed as showing 99 per cent of agreement, a totally misleading figure. Suppose, however, that one has the bloods from 1,000 syphilitics. A given method finds 900 of these bloods positive; another test finds only 600 of these bloods positive. The accuracy of the first method is 90 per cent, whereas the accuracy of the other method is only 60 per cent. It is, therefore, manifest that in preparing statistics on the adequacy of a method, by comparison with another test, one must have, as a basic figure, the number of persons known to be infected. The efficacy of any method must be determined primarily on the basis of the number of actual syphilitic patients, and secondarily on a control group consisting of a sufficient number of nonsyphilitic persons to demonstrate that the test gives no nonspecific reactions, or, at most, only an occasional one.

FAITHFUL EXECUTION OF AN AUTHOR'S TECHNIC

Having chosen a method because of belief in its adequacy, as manifested by its sensitiveness, specificity, and dependability, it is incumbent upon the worker to execute each step of the test in the exact manner prescribed by the

author. There are several reasons for this caution, which might perhaps better be called "admonition." Before publication of his test, the author has carried on hundreds of experiments to perfect his technic. His directions and instructions are based on the technic he has found most successful, and no change should be made except on the basis of equal or more painstaking research. I recently read an abstract of an article on the Hinton test which stated that the sole advantage of the test was that it was easier to perform, but that it was no more sensitive than the Wassermann test. There was, however, a statement in the article that 0.1 c.c. and 0.3 c.c. of serum had been used in the Hinton test, instead of 0.3 c.c. and 0.5 c.c. of serum as directed. In my experience, fully 30 per cent of the specimens that give a well-marked characteristic reaction with 0.5 c.c. of serum are negative when only 0.3 c.c. is used. My selection of 0.3 c.c. and 0.5 c.c. was based on an examination of several thousands of bloods of persons who were investigated clinically as well as serologically.

Besides strict adherence to technical details, each worker must remember that as sensitiveness of a method increases there must be increasing precision in its execution. The Hinton test, which has proved, in my hands, more than twice as sensitive as the Wassermann test, is perceptibly affected by slight differences in the temperature of incubation, whereas the same temperature variations have had no discernible effect on the Wassermann test. In diluting antigen for the Wassermann test, I have not been able to discover any marked difference in the result when slight variations of the method have been made. In the Hinton test, however, in three laboratories under my direction, the slightest variations in diluting the indicator (I prefer to call it indicator rather than antigen) have caused inconsistencies in the results. Several months ago I designed a special flask[3] for the purpose of diluting indicators, and as a consequence the three laboratories almost invariably obtained the same results when the same specimens of blood were tested.

I have recently simplified the technic of the Hinton test (third modification).[4] This simplified method, which has been in operation in my laboratories for the past six months, is being maintained because it is superior in sensitiveness and specificity to my former methods. Its description, according to the statement of my students, sounds difficult and austere, but after they have actually performed the test their response is that it is very simple. The reason why the test sounds difficult is that I have made the directions as explicit as I could; each admonition represents a pitfall, the discovery of which has occupied days, weeks, months, or in some instances even years.

SUMMARY

The chief value of a serologic method of testing for syphilis lies in a high degree of sensitiveness, specificity, and dependability; the ease of execution and interpretation, as well as economy in cost, are secondary qualifications. Flocculation methods fulfill these requirements best. Of those now available, choice must be made on the basis of accurate, but not misleading, statistical

studies, rather than on simple comparisons with the Wassermann or any other test. Whatever method is chosen, best results will almost invariably follow from the closest adherence to the author's directions.

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STUDIES ON HEMOLYTIC STREPTOCOCCI ISOLATED FROM HEMORRHAGIC SMALLPOX*

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POSSIBLY no group of bacteria can claim more varied and more numerous types of pathogenic activity than the streptococci. Although these microorganisms are easily recognized, because of their morphology, the possibilities of their unwarranted behavior are always a source of interest to the research worker in these fields.

The hemolytic streptococci are responsible for inflammatory and suppurative conditions which vary greatly in severity and effect. Such lesions range from trivial localized inflammations of transitory duration to those of great intensity, spreading rapidly to the tissue spaces by way of the blood. Exogenous infection, associated sometimes with epidemic spread, is a conspicuous feature of certain diseases due to the hemolytic streptococci, and virulent strains may produce sudden and fatal septicemia without any apparent lesion at the site of the initial infection. Following Jenner's[1] notable work on smallpox, there was a series of attempts to prove the bacterial origin of variola, and the literature on the contents of vesicles is large and embodies a variety of opinions. Many bacteria have been described in this connection, some of them new, others old and well-known forms. Among the researches in this field, we find the works of Garre,[2] Protopopoff,[3] Waele and Sugg,[4] Perkins and Pay,[5] and Hines,[6] dealing with the various phases of smallpox and their relations to the streptococci.

Because of these facts the present studies have been undertaken, not to prove the organism in question to be the etiologic factor, but rather to study its cultural, morphologic, serologic, and immunologic characteristics, and thus classify the different strains by their reactions to the several stimuli.

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CULTURAL HISTORY

In the course of a rather severe epidemic of smallpox which occurred in Detroit several years ago (1924), the opportunity presented itself to study, culturally, fatalities, the result of hemorrhagic smallpox. The epidemic was widespread and the individuals observed were from various parts of the city. Each patient entered the hospital as a confluent type and the hemorrhagic developments appeared from four to eight days after admittance. The strains when cultured on blood agar were hemolytic, and when examined macroscopically, the colonies were similar. In all, 9 strains were isolated and for purposes of identification have been designated as "Var.," while those forms from scarlet fever, erysipelas, and puerperal septicemia have been identified by number only.

All cultures isolated from sources of fatal hemorrhagic smallpox were from adults, ranging in age from twenty-eight to forty years, none of whom gave any history of vaccination. Pure cultures were obtained in every instance although this fact is not stressed as being of significance as an etiologic factor. Strains isolated from puerperal septicemia were obtained from the blood of patients who later died. Streptococci obtained from erysipelas were in one instance (02168), a blood culture from a fatal terminus, and in the other (02005) a culture from a skin lesion. Both of these strains show high cross agglutinating titer with the Birkhaug strains. Those streptococci isolated from authenticated instances of scarlet fever were from throat cultures and both strains produce specific toxin of a high titer.

In the isolation of streptococci one requisite is that the culture medium be fresh and that its composition be such that the organism in question will grow satisfactorily. A great variety of both fluid and solid mediums have been advocated by research workers, most of them making use of tissue extracts of brain, kidney, or spleen. Previous experiments in the isolation of streptococci led to the conclusion that veal broth and the brain liver media of Hibler gave most satisfactory results. The standard veal broth was prepared, containing the aqueous extractives of 500 gm. of lean chopped veal, 5 gm. of c. p. sodium chloride, and 10 gm. of Witte's peptone per liter. Dextrose in the amount of 0.2 per cent was added. The final reaction was adjusted to P_H 8.0 and the broth distributed into 500 c.c. flasks, each containing 200 c.c. The tissue medium of von Hibler[7] as modified by Goss, Barbarin and Haines[8] was also employed in making blood cultures.

The blood was drawn at the bedside under aseptic conditions from the median basilic vein and transferred immediately into culture medium. A quantity of 2 c.c. was added to the veal infusion bouillon and a like amount to the flasks containing the Hibler medium. The seeded flasks were taken at once to the laboratory and 1 c.c. of the diluted blood added to a mixture of 5 per cent sterile rabbit blood and 2 per cent agar, thoroughly agitated and poured into sterile Petri dishes. The resulting mixture represented a 1:100 dilution of the original blood. The plates, veal bouillon, and the Hibler medium were then incubated for twenty-four hours at 37° C. after which they were examined macroscopically.

In every instance a pure culture of *Streptococcus hemolyticus* was obtained from both the fluid and solid mediums. The blood plates never showed less than 400 and in several instances the number was as large as 900 organisms per c.c., computed on the basis of the original sample. Well isolated, typical single

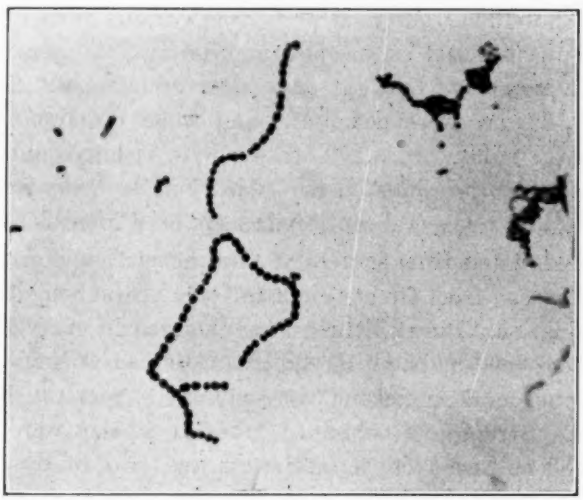

Fig. 1.—Cultures from blood of individuals suffering from hemorrhagic smallpox. Both solid and liquid mediums. Strain Var. 8, twenty-four-hour bouillon culture. G. violet. ×1500.

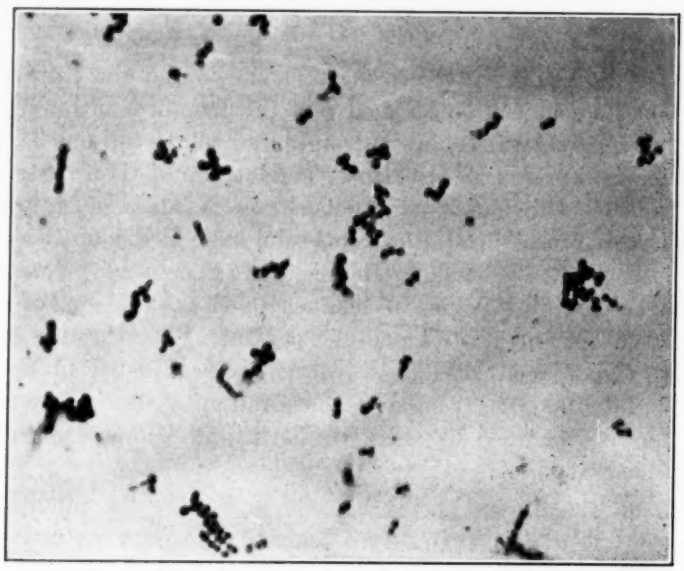

Fig. 2.—Cultures from blood of individuals suffering from hemorrhagic smallpox. Both solid and liquid mediums. Strain Var. 8, twenty-four-hour blood agar culture. G. violet. ×1500.

colonies, appearing on the blood plates, were selected and subcultures were made on blood agar slants, veal bouillon, and whole defibrinated sheep blood.

MORPHOLOGIC AND CULTURAL CHARACTERISTICS

The bacteriologic literature is crowded with classifications of organisms of every sort, based usually upon their metabolic activities, morphology, pathogenic

properties, manner of growth, and their ability to produce toxins, either soluble or insoluble. Numerous tests have been devised by thoughtful investigators to determine the type of organism under consideration. There occur, however, many criticisms of these classifications and as yet no general agreement has been reached relative to their respective values. In view of the various methods of classification and the apparent dissention among workers in this field as to their merits, it is not the intention of the author to attempt to classify the organisms

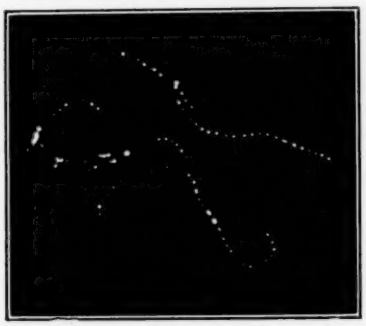

Fig. 3.—Cultures from blood of individuals suffering from hemorrhagic smallpox. Both solid and liquid mediums. Strain Var. 3, twenty-four-hour bouillon culture. Negative strain. ×1500.

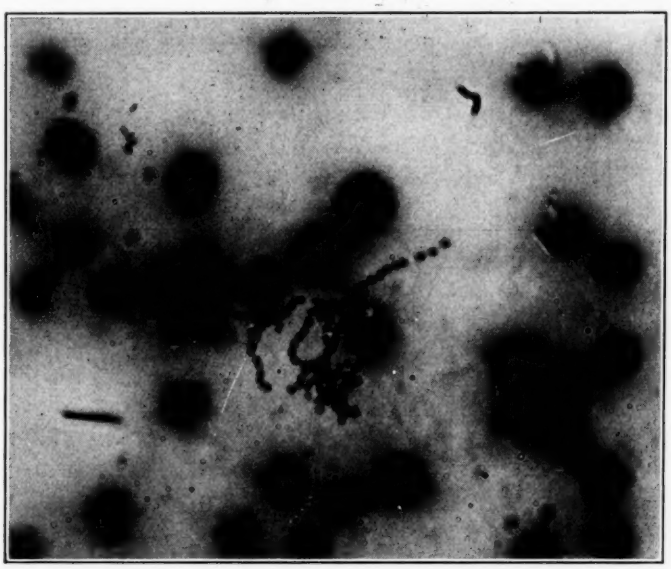

Fig. 4.—Cultures from blood of individuals suffering from hemorrhagic smallpox. Both solid and liquid mediums. Strain Var. 3, blood smear from rabbit given 2 c.c. of a bouillon culture. Wright's stain. ×1500.

in question by any certain concise methods, but to give a true picture of the reactions on different culture mediums according to the various workers.

The growth and morphology of each strain of *Streptococcus variolae* isolated, and cultures of streptococci from scarlet fever, erysipelas, and puerperal septicemia, were carefully noted, when cultured on infusion broth and blood agar. The data which are presented in Table I seem to indicate that there is but little variation between the strains.

TABLE I

MORPHOLOGIC AND CULTURAL CHARACTERISTICS OF STREPTOCOCCI ISOLATED FROM SMALLPOX, SCARLET FEVER, ERYSIPELAS, AND PUERPERAL SEPTICEMIA

CULTURE	GROWTH		MORPHOLOGY		HEMOLYSIS	BILE SOL.
	BLOOD AGAR	BROTH	BLOOD AGAR	BROTH		
Var. 1	Small, smooth white colony	Clear, flaky sediment	Cocci in pairs and chains	Chains of 10 to 50	Wide zone	Negative
Var. 2	Small, smooth white colony	Clear, flaky sediment	Small cocci in pairs and chains	Chains of 6 to 50	Medium zone	Negative
Var. 3	Small, smooth white colony	Clear, flaky sediment	Small cocci in short chains	Chains of 10 to 50	Wide zone	Negative
Var. 5	Small, smooth blue white colony	Clear, flaky sediment	Medium cocci in short chains	Chains of 10 to 50	Wide zone	Negative
Var. 6	Small, smooth blue white colony	Clear, flaky sediment	Medium cocci in short chains	Chains of 8 to 50	Wide zone	Negative
Var. 7	Small, smooth white colony	Clear, flaky sediment	Small cocci in pairs and chains	Chains of 8 to 60	Wide zone	Negative
Var. 8	Small, smooth white colony	Clear, flaky sediment	Cocci in pairs and chains	Chains of 6 to 50	Wide zone	Negative
Var. 12	Small, smooth white colony	Clear, flaky sediment	Cocci in short chains	Chains of 10 to 50	Medium zone	Negative
Var. 14	Small, smooth opaque colony	Clear, flaky sediment	Medium cocci in short chains	Chains of 6 to 40	Wide zone	Negative
Ery. 02168	Small, smooth white colony	Semi cloudy with sediment	Small cocci in short chains	Chains of 3 to 15	Narrow zone	Negative
Ery. 02005	Small, smooth white colony	Semi cloudy with sediment	Small cocci in short chains	Chains of 6 to 20	Narrow zone	Negative
Scar. 01730	Small, smooth colony	Clear, flaky sediment	Medium cocci in chains	Chains of 6 to 30	Wide zone	Negative
Scar. 01841	Small, smooth colony	Clear, flaky sediment	Medium cocci in short chains	Chains of 6 to 22	Narrow zone	Negative
Puer. 02224	Small, smooth colony	Semi cloudy flaky sediment	Medium cocci in short chains	Chains of 6 to 30	Wide zone	Negative
Puer. 02087	Very small, smooth colony	Clear, flaky sediment	Medium cocci in short chains	Chains of 4 to 30	Wide zone	Negative

It appears from Table I that all the strains isolated from hemorrhagic smallpox were, in the main, very similar and indistinguishable culturally from other hemolytic streptococci, such as those from scarlet fever, puerperal septicemia, and erysipelas. They produce small, smooth colonies with hemolytic zones of varying diameters. Grown in veal bouillon they have a tendency to settle to the bottom in a flaky sediment.

The cells in strains 01841 and 01730, isolated from scarlet fever, were somewhat smaller than those isolated from the patients with variolae. In general, the strains from hemorrhagic smallpox produced a much wider zone of hemolysis than the others studied.

Figs. 1 to 4 show photomicrographs of 2 strains of streptococci isolated from smallpox cultured on solid medium, in bouillon, and also a direct blood smear from a rabbit.

FERMENTATION REACTIONS

Although the many authors who have carried out research on the fermentation reactions of the different streptococci are at variance in their opinions relative to the merit of this test, there is no doubt that characteristics thus established for the many strains of these organisms serve as a valuable aid in differentiating between the predominant types. Comparatively few have considered all criteria in classifying the strains studied by them. The Germans have given special attention to the morphology of the streptococci and their appearance on blood agar; the English have made an exhaustive study of the fermentation reactions of these organisms, while the Americans have divided their attention between one or the other set of characters. It is obvious that any research on the subject of the fermentation reactions of the streptococci must take into consideration certain essential precautions if reliable results are to be obtained. In these tests special care was exercised in the selection of the test substances in order that they be pure, sufficient time for the fermentation was allowed to elapse, and none of the test substances was injured by overheating.

All strains of the streptococci tested were first grown for 10 generations in veal infusion bouillon, P_H 8.0, and tested daily for purity. They were then transplanted for 3 generations on blood agar after which they were transferred to the carbohydrate medium. Double strength broth was made sugar-free in the usual manner, using *B. coli* as the fermentating agent. After complete fermentation the broth was filtered through a sterile Berkefeld filter, adjusted to P_H 8.0 and to every 200 c.c. of the sugar-free broth, 4 c.c. of Andrade's indicator, together with 4 c.c. of the test substance, was added. This was sterilized in flowing steam for twenty minutes on three successive days, cooled, and 200 c.c. of serum water (1 part horse serum to 300 parts of sterile distilled water) added under aseptic conditions. The finished carbohydrate serum broth was then tubed and incubated several days to insure sterility. Acid production caused by the fermentation of the test substance was noted by the color change and recorded. Table II shows the results of the fermentation action of the different strains of streptococci being studied.

It will be noted that with one exception all organisms isolated from smallpox are negative for lactose, while the control strains from erysipelas, scarlet

TABLE II

FERMENTATION REACTIONS OF THE STREPTOCOCCI FROM SMALLPOX, SCARLET FEVER, ERYSIPELAS, AND PUERPERAL SEPTICEMIA

CULTURE	SOURCE	SACCHAROSE	DEXTROSE	MANNITE	LACTOSE	SALICIN	INULIN	LEVULOSE	D-MANOSE	RAFFINOSE	XYLOSE	MALTOSE	CONTROL
Var. 1	Sm. Px.	+	+	−	−	+	−	+	+	−	−	+	−
Var. 2	Sm. Px.	+	+	−	−	+	−	+	+	−	−	+	−
Var. 3	Sm. Px.	+	+	−	−	+	−	+	+	−	−	+	−
Var. 5	Sm. Px.	+	+	±	+	+	−	+	+	−	−	+	−
Var. 6	Sm. Px.	+	+	−	−	+	−	+	+	−	−	+	−
Var. 7	Sm. Px.	+	+	−	−	+	−	+	+	−	−	+	−
Var. 8	Sm. Px.	+	+	−	−	+	−	+	+	−	−	+	−
Var. 12	Sm. Px.	+	+	−	−	+	−	+	+	−	−	+	−
Var. 14	Sm. Px.	+	+	−	−	+	−	+	+	−	−	+	−
01730	Scar.	+	+	−	+	+	−	+	+	−	−	+	−
01841	Scar.	+	+	−	+	+	−	+	+	−	−	+	−
02168	Ery.	+	+	−	+	+	−	+	+	−	−	+	−
02005	Ery.	+	+	−	+	+	−	+	+	−	−	+	−
02224	Puer.	+	+	−	+	+	−	+	+	−	−	+	−
02087	Puer.	+	+	−	+	+	−	+	+	−	−	+	−

fever, and puerperal septicemia all ferment lactose. This action, together with the action on the other test substances, would place the streptococci from smallpox under the head of *Streptococcus equi,* according to Holman's classification. The six comparative strains, would according to the same scheme, fall into that group, usually referred to as *Streptococcus pyogenes.* However, a careful review of numerous authors on the subject of *Streptococcus equi* shows such a variance of opinions that one is at a loss to know the true characteristics of the organism in question. In addition, each culture was planted in litmus milk and observed during a period of two weeks. The data are presented in Table III.

TABLE III

ACTION OF THE STREPTOCOCCI ON LITMUS MILK

CULTURE	SOURCE	ACIDITY	COAGULATION	REDUCTION
Var. 1	Sm. Px.	−	−	−
Var. 2	Sm. Px.	−	−	−
Var. 3	Sm. Px.	−	−	−
Var. 5	Sm. Px.	Faint, 14 hr.	+	−
Var. 6	Sm. Px.	−	−	−
Var. 7	Sm. Px.	−	−	−
Var. 8	Sm. Px.	−	−	−
Var. 12	Sm. Px.	−	−	−
Var. 14	Sm. Px.	−	−	−
02168	Ery.	+	−	−
02005	Ery.	+	−	−
01730	Scar.	+	−	−
01841	Scar.	+	−	−
02224	Puer.	+	−	−
02087	Puer.	+	−	−

From Table III we find that those strains of streptococci isolated from smallpox show no acid production in litmus milk while the comparative strains

all show marked acid production in twenty-four hours. This reaction according to Jones[9] and Mathers,[10] is characteristic of *Streptococcus equi* but is contradicted by the work of Besson[11] and Bergey.[12] Such opinions make a definite classification impossible. The fact that the strains of streptococci from smallpox vary among themselves and from the strains used as controls in their ability to ferment certain differential substances and their action on litmus milk, does indicate, however, that the strains in question are different from those usually classed as *Streptococcus pyogenes.*

VIRULENCE

The most significant property possessed by certain of the streptococci is that of dissolving red blood cells. The fact that this hemolytic action is confined to certain types of streptococci and not possessed by others serves as a method of classification and to some extent, bears a definite relation to the virulence of the organism. A review of the literature including the works of Rolly,[13] Besson,[11] and McLeod,[14] shows a difference of opinion, relative to the direct relation between hemolysin production and virulence.

In order that the virulence of the strains be at a maximum, they were serially transferred at twenty-four-hour intervals for 10 generations in 5 per cent horse serum broth, P_H 8.0. The culture representing the tenth generation was then injected into white mice which were subsequently killed. The heart blood was then transferred into tubes of serum broth and after twenty-four-hour incubation at 37° C., a portion of the bouillon was injected into a second

TABLE IV

VIRULENCE TESTS. HEMOLYTIC STREPTOCOCCI ISOLATED FROM SMALLPOX

CULTURE	MOUSE	AMOUNT	RESULT
Var. 1	1	1.0	Died, 48 hr.
	2	0.1	Died, 72 hr.
	3	0.01	Lived
Var. 2	1	1.0	Lived
	2	0.1	Lived
	3	0.01	Lived
Var. 3	1	1.0	Died, 24 hr.
	2	0.1	Died, 72 hr.
	3	0.01	Died, 72 hr.
	4	0.001	Lived
Var. 5	1	1.0	Lived
	2	0.1	Lived
	3	0.01	Lived
Var. 6	1	1.0	Died, 72 hr.
	2	0.1	Died, 72 hr.
	3	0.01	Lived
Var. 7	1	1.0	Died, 48 hr.
	2	0.1	Lived
	3	0.01	Lived
Var. 8	1	1.0	Died, 24 hr.
	2	0.1	Died, 72 hr.
	3	0.01	Lived
Var. 12	1	1.0	Died, 24 hr.
	2	0.1	Died, 48 hr.
	3	0.01	Lived
Var. 14	1	1.0	Died, 48 hr.
	2	0.1	Died, 24 hr.
	3	0.01	Lived

mouse. This technic was repeated until the virulence of each strain was at a maximum. Table IV contains the results of these experiments.

It would appear from Table IV that no strain of the streptococci isolated from smallpox is of high virulence for mice. Only 1 strain killed in a 1:100 dilution and the remaining strains killed only in a 1:10 dilution. It is to be noted that these strains are of a relatively low virulence when compared with those killing mice in dilutions of 1:100,000.

AGGLUTINATION EXPERIMENTS

The various immunity reactions so frequently employed for diagnostic purposes, such as agglutination, complement fixation, and bacteriotropine tests, give the most convincing evidence of biologic grouping. Many strains of bacteria, although indistinguishable by cultural methods, may be divided into separate groups. In the study of the hemolytic streptococci there seems from the beginning, some basis for an immunologic grouping of these organisms.

The immune serums used in the serologic tests were obtained by the immunization of rabbits weighing approximately 2,000 gm. Previous experience, Ferry and Fisher,[15] showed that individual rabbits immunized intravenously gave less variation and more constant results than those immunized by other methods. Each animal was given 3 intravenous injections of killed germs suspended in saline, preceded by one desensitizing injection, given subcutaneously. Five days after the last injection the rabbits were bled. The first, or desensitizing injection, given subcutaneously was 0.5 c.c. followed by 1 of 1 c.c. and 2 of 2 c.c. each, given intravenously three days apart. Two rabbits were treated with each of the 15 strains of streptococci being studied, after tests for agglutinins had been made against scarlet fever streptococcus 01730, puerpural septicemia 02087, erysipelas 02168, and smallpox Var. 3 and Var. 8. No rabbit was used whose serum showed any degree of agglutination in amounts less than 1:20 dilution. The suspensions to be used as antigens were grown in 100 c.c. quantities in a 0.1 per cent glucose broth, P_H 8.0, for twenty-four hours. The cultures were then centrifugalized and the sediment washed with saline to prevent the production of agglutinins to the protein constituents of the medium. The sediment was then taken up with normal saline solution containing 0.4 per cent tricresol and standardized in such a manner as to correspond to a suspension of 1,000,000,000 organisms per c.c. as this was found to be a safe dose for rabbits and compares closely to the count of broth cultures.

Agglutination tests may meet with difficulties on account of the tendency of streptococci to form clumps. The tendency of the streptococci isolated from smallpox to show spontaneous agglutination was overcome by using care in the preparation of the organisms to be used in the agglutination reaction. The broth was made from selected meat, and in place of the usual sodium chloride a sufficient quantity of dibasic sodium phosphate mixture was added to give the desired isotonicity and P_H. The organisms were removed from the culture medium by centrifugation and washed in broth prepared in the manner previously described. They were resuspended in the same type medium in approximately the concentration that was used in the agglutination reaction. The clumps were broken down by vigorous shaking and 0.5 per cent solution of

formalin added to kill the organisms. The suspensions of the controlling strains, while showing less tendency toward spontaneous agglutination, were prepared in the same manner as were the strains isolated from smallpox.

The serums employed in the tests were made up to the various concentrations by dilution with the same broth used for suspending the bacteria. Each reaction was controlled by a series of both normal serum, and suspension in the absence of serum. Equal quantities of dilute serum and bacterial suspensions were added to each tube and placed at 37° C. for eighteen hours. If this procedure is carefully followed there is less chance of nonspecific granulation occurring. If clumping develops in the broth control or in more than the first few normal serum controls, the test must be discarded.

Homologous and cross agglutinations were run with the 9 strains of smallpox streptococci and 2 each of scarlet fever, puerperal septicemia, and erysipelas streptococci. Table V gives the highest dilution of a specific serum showing definite agglutination against its homologous and heterologous strains. The 9 strains of streptococci from smallpox did not cross agglutinate to any appreciable extent with those from scarlet fever, puerperal septicemia, or erysipelas. There was, however, cross agglutination in high dilutions between the several strains from smallpox.

COMPLEMENT FIXATION REACTIONS

The principle of complement fixation has long been utilized in bacteriologic investigations, for determination, in the serum, of specific antibodies. Although a classification of streptococci on the basis of complement fixation has not proved as valuable as agglutination tests, it has been employed by many investigators to establish a natural classification of these organisms. In these studies, homologous and cross complement fixation tests were carried out with the same cultures as those used for agglutination, with three exceptions, due to the anticomplementary action of antigens prepared from Var. 5, Var. 8, and 02005.

Antigens were prepared in the following manner: Each culture was grown for twenty-four hours on 0.2 per cent dextrose sheep serum agar, P_H 8.0, in Roux flasks. The growth was washed off in 15 c.c. of normal saline solution and made up to 50 c.c. per flask. Each suspension was shaken for fifteen minutes and the remaining clumps allowed to settle for several hours. The supernatant fluid, containing the organisms was then decanted off, heated to 56° C. for one hour and killed with 0.5 per cent phenol. Stable antigens of all cultures, with the noted exceptions, were prepared by this method. They were nonhemolytic in quantities of 0.5 c.c. of a 1:5 dilution, nonanticomplementary in workable units, and comparatively high in antigenic value. The amount of antigen used was from 2 to 4 times the antigenic unit, usually 4 times, depending on the anticomplementary unit.

The tests were made by an adaptation of the Kolmer[16] technic, the quantitative determinations being carried out with decreasing amounts of inactivated immune serum. Referring to Table VI one finds the results of the complement fixation studies. The average of the highest dilutions of the two rabbits immunized with a specific strain is taken as an index in each instance. A comparison of these results shows that they run about parallel with those of the agglutination tests.

TABLE V

AGGLUTINATION RESULTS WITH STREPTOCOCCI ISOLATED FROM SMALLPOX, SCARLET FEVER, ERYSIPELAS, AND PUERPERAL SEPTICEMIA

HIGHEST DILUTION OF SERUM SHOWING AGGLUTINATION AGAINST

ANTISERUM FOR	STREPTOCOCCI FROM SMALLPOX									SCARLET FEVER		ERYSIPELAS		PUERPERAL SEPTICEMIA	
	VAR. 1	VAR. 2	VAR. 3	VAR. 5	VAR. 6	VAR. 7	VAR. 8	VAR. 12	VAR. 14	01730	01841	02168	02005	02087	02224
Var. 1	3200	2400	600	2400	2800	2800	1400	2400	2000	20	20	200	20	400	200
Var. 2	1800	2800	400	1800	1800	1600	2400	1800	2000	20	20	20	20	20	200
Var. 3	2000	2400	1600	2000	1800	2800	2000	2400	2000	20	20	200	20	20	20
Var. 5	1600	1400	400	2800	1600	2400	1800	1600	1600	200	20	20	20	20	20
Var. 6	2400	2000	800	2400	3200	2800	1600	2400	2800	20	20	200	20	20	200
Var. 7	2000	1800	600	2400	2800	3200	2400	1800	2000	200	20	20	20	200	200
Var. 8	2400	2000	200	2400	3200	2800	2400	1600	2000	200	20	200	20	20	20
Var. 12	2000	1800	200	2000	1600	2400	2400	3200	2000	200	200	20	20	20	20
Var. 14	2000	2000	400	1600	1800	2800	2000	2800	3200	20	20	20	20	20	20
01730	20	200	200	20	20	20	200	200	20	2800	2400	1800	1800	1800	2000
01841	20	20	20	200	200	200	20	20	20	2400	2400	1800	1800	2000	1600
02168	20	200	20	20	20	20	200	200	20	2000	1000	2800	2000	2400	2400
02005	200	20	20	20	20	20	20	20	20	2400	2000	2800	2800	2400	2000
02087	20	20	20	20	200	20	20	20	20	1800	1000	1600	1800	2800	2400
02224	20	20	20	20	20	20	200	20	200	1400	1400	1600	1400	2400	2800

TABLE VI

COMPLEMENT FIXATION WITH THE STREPTOCOCCI

HIGHEST DILUTION OF SERUM SHOWING FIXATION AGAINST ANTIGENS OF

ANTISERUM FOR	STREPTOCOCCI FOR SMALLPOX							SCARLET FEVER		ERYSIPELAS	PUERPERAL SEPTICEMIA	
	VAR. 1	VAR. 2	VAR. 3	VAR. 6	VAR. 7	VAR. 12	VAR. 14	01730	01841	02168	02087	02224
Var. 1	800	600	80	1000	600	800	800	80	60	50	60	60
Var. 2	800	1000	80	600	500	500	600	80	80	60	60	60
Var. 3	600	600	200	500	800	600	800	60	80	60	60	60
Var. 5	500	400	80	600	1000	600	600	60	60	60	60	60
Var. 6	600	500	80	1000	800	600	800	60	60	50	50	60
Var. 7	800	600	100	800	2000	600	800	60	50	60	50	50
Var. 8	600	500	80	500	600	200	500	60	50	60	80	60
Var. 12	800	800	80	600	800	1000	800	80	100	60	60	80
Var. 14	800	800	80	800	800	400	600	60	80	60	60	80
01730	60	60	50	50	60	80	60	1000	1000	600	600	500
01841	60	80	60	50	80	60	50	800	800	600	600	800
02168	60	80	50	80	60	60	50	800	400	1000	600	800
02005	60	60	50	60	60	60	60	600	500	400	500	500
02087	60	80		60	60	60	80	600	600	600	1000	600
02224	60	30	60	60	80	60	60	600	800	800	800	1000

TOXIN PRODUCTION

During the last decade an exhaustive study of the streptococci and their soluble toxins has occupied a major portion of the time of many workers. In spite of extensive research upon the nature of the poisons produced by these microorganisms, our knowledge is still incomplete. The grave systemic symptoms often accompanying streptococcus lesions argue strongly for the production, by these organisms, of a powerful, diffusable, toxic substance. The toxins produced by the streptococci differ from most other true bacterial toxins in being thermostable. Many preparations will withstand boiling, and some may be boiled for an hour without entirely losing their capacity for skin reactions. Inasmuch as laboratory animals are not highly susceptible to the toxin, its standardization is limited at present to tests on susceptible human beings.

The question as to whether the streptococci isolated from hemorrhagic smallpox would produce a soluble toxin, thus presented itself as worthy of consideration. Was the fatal outcome of these cases due to a bacteremia, to a toxemia, or to a combination of the two? The toxin production was carried out on especially prepared medium. Veal infusion agar was made from carefully selected meat, and great care was used to remove all fat from the broth. One per cent Witte peptone, 0.85 per cent sodium chloride, c. p. were used and the broth titered to P_H 7.8. The medium was placed in 200 c.c. quantities and seeded with the various strains of streptococci isolated from smallpox and with one strain each of scarlet fever, erysipelas, and puerperal septicemia. Incubation at 37° C. was carried on for six days when the cultures were filtered through sterile Berkefeld V filters and the filtrate preserved with 0.4 per cent phenol. Sterility determinations in bouillon and tests on guinea pigs were carried out to determine the safety of the product.

The cutaneous reactions were done at the Wayne County Training School, the individuals tested ranging in age from eight to sixteen years with an average

of twelve years. Both sexes were represented, although males predominated. Only those reactions which were positive to strongly posititve were accepted and reactions under 1 cm. in diameter were regarded as negative.

TABLE VII

CUTANEOUS TESTS WITH TOXINS OF THE STREPTOCOCCI

	LEFT ARM				RIGHT ARM			
	VAR. 1 1:300	VAR. 3 1:300	VAR. 7 1:300	VAR. 8 1:300	01730 1:2000 SCAR.	02168 1:250 ERY.	02224 1:300 PUER.	CONTROL 1:250 HEATED
1. Y, R.	1.5×1.5		1×1	1×1		2×2	2×2	
2. R, E.		2×2	1×1			2×2		
3. J, K.							1×1	
4. P, M.	1.5×1.5	1×1	1×1	1×1		2×2	1.5×1.5	
5. S, K.						2×2	1×1	
6. S, M.						2×2	1.5×1.5	
7. B, D.			1×1	1×1	2×2	1×1	2×1.5	
8. A, H.		1.5×1.5	2×2	2×2	1×1	2×2	1.5×1.5	
9. M, M.						2.5×2.5	1.5×1.5	
10. L, R.	1×1	1.5×1.5	2×2	1×1	1.5×2	2.5×2	2.5×2	
11. S, E.								
12. K, L.				1×1		1×1	1×1	
13. Z, I.	1×1	1×1				1×1	1×1	1×1
14. A, S.	1×1	1×1	1×1	1×1		1.5×1.5	1×1	
15. D, W.						1×1	1×1	
16. A, A.		1×1	1.5×1.5	1×1		1.5×1.5	1.5×1.5	
17. A, G.	1×1		1×1	1×1	2×2	3×2	2×2	
18. J. H.		1×1				1×1	1×1	
19. J, E.	1×1	1×1		1×1		1×1	1×1	
20. P, A.	2×2	3×3	2×2	3×3		2×2	2×2	
21. F, C.	1×1	2×2	2×2	2×2	2×2	2×2		
22. H, A.								
23. F, J.								
24. D, R.	2×2	3×3	2×2	2×2	2.5×2	2×2		
25. D, T.		1×1	1×1		1×1	1×1		
26. D, G.		2×2	1×1	1×1	1×1			
27. H, O.		1×1		1×1	1×1	1×1		
28. B, H.								
29. S, E.		2×2			1×1			
30. M, J.		1×1	1×1	1×1	1×1	1.5×1.5		
31. S, L.	1.5×1.5	2×2	2×2	2×2	1×1	1×1		
32. K, N.	1.5×1.5	1.5×1	1.5×1	1×1	1.5×1.5	1.5×2		
33. S, J.					1×1			
34. K, G.		1×1	1×1	1×1	1×1	1×1		
35. M, J.								
36. M, M.					1.5×1.5	1.5×1.5		

The results of skin tests with the toxins of 8 strains of streptococci isolated from hemorrhagic smallpox and 1 each of the comparative strains, may be seen in Table VII. The control toxin in each instance was heated at 100° C. for thirty minutes in the autoclave. Varying dilutions of the filtrates were made, and subcutaneous injections, given on the forearm of the individuals tested, were read in twenty-four hours. Dilutions were made so that 1 c.c. of the original filtrate would contain 3,000 skin test doses. The toxins from 01730, scarlet fever, 02168 erysipelas, and 02224 puerperal septicemia, had all been previously neutralized with convalescent serum and also by horse antitoxin produced by injection of organisms from scarlet fever (Dick), erysipelas (Birkhaug), and puerperal septicemia (Birkhaug).

TABLE VIII

SHOWING NEUTRALIZATION POWER OF STREPTOCOCCUS (SMALLPOX) ANTITOXIN

	LEFT ARM				RIGHT ARM				
	TOXIN VAR. 8 DIL. 1:300	TOXIN VAR. 8 HEATED DIL. 1:300 (CONTROL)	ANTITOXIN DIL. 1:10 (CONTROL)	TOXIN VAR. 8 DIL. 1:150 PLUS EQ. PARTS ANTITOXIN DIL. 1:10	TOXIN VAR. 8 DIL. 1:150 PLUS EQ. PARTS NORMAL SERUM DIL. 1:10	TOXIN VAR. 8 DIL. 1:150 PLUS EQ. PARTS ANTITOXIN DIL. 1:30	TOXIN VAR. 7 DIL. 1:300	TOXIN VAR. 7 DIL. 1:150 PLUS EQ. PARTS ANTITOXIN DIL. 1:10	
1. C, J.	1 × 1				1 × 1.5		1 × 1		
2. D, J.	2 × 1.5		2.5 × 2	2 × 1	2 × 1.5	2 × 2	1.5 × 1.5	2 × 2	Serum
3. B, E.	1 × 1				1 × 1		2.5 × 2.5		
4. Z, E.	1 × 1				1 × 1	1 × 1	1 × 1		
5. K, R.	2 × 2	1 × 1		1 × 1.5	1 × 1	1 × 1.5	1.5 × 1	1 × 1	Protein
6. K, A.	2 × 2				1.5 × 2		2 × 2		
7. P, J.	2 × 2		2 × 2.5	2 × 2.5	2 × 2	2 × 2.5	3 × 3	2 × 2.5	Serum
8. A, P.	2 × 2.5				2 × 2	1 × 1	2.5 × 2.5		
9. D, M.	1 × 1				1 × 1		1 × 1		
10. K, M.							1 × 1.5		
11. R, D.							1 × 1		
12. K, J.	1.5 × 2.5		1 × 1	1 × 1	1 × 1	1 × 1	1 × 1	1 × 1	Serum

A study of the table would seem to indicate some relationship between the smallpox strains and those of the streptococci from erysipelas and puerperal septicemia. Out of a total of 79 individuals tested, only 14 gave reactions to the erysipelas and puerperal septicemia toxins and no reaction to the smallpox toxins. Individuals reacting to the puerperal septicemia and erysipelas toxins and one or more strains of the smallpox toxins numbered 45. The results would also indicate that the smallpox strains are closely related in their ability to produce soluble toxins.

In order to prove conclusively that the substance causing the reactions in the intradermal tests was a true toxin, an effort was made to produce an antitoxin. The streptococcus immune serum or antitoxin would thus neutralize its own toxin when mixtures of toxin and antitoxin were injected intradermally into an individual who was susceptible to the toxin. The immune serums were produced by repeated intravenous injections of undiluted filtrate into rabbits. The first injection was 3 c.c. of filtrate followed every third day by increasing amounts until a maximum of 10 c.c. was given. The animals were bled ten days after the last injection. A control serum was obtained from nonimmunized (normal) rabbits. Equal amounts of diluted filtrate and a 1:20 dilution of both immune and nonimmune serums were mixed and allowed to incubate for one hour at 37° C. Varying dilutions of the antitoxin were used in the test. Persons who were positive to the toxin of one strain of the streptococci from smallpox were selected and injected as shown in Table VIII.

Individual variations in the persons injected account for several unwarranted reactions. Two of the group were sensitive to the serum while two others reacted to the protein. The antitoxin in a dilution of 1:10 neutralized the toxins from Var. 7 and Var. 8 although a 1:20 dilution showed no neutralization in 2 instances.

SUMMARY

From the results of this work it would seem apparent that certain differences exist between the hemolytic streptococci isolated from blood cultures of fatal hemorrhagic smallpox and the pyogenic type of organism of scarlet fever, erysipelas, and puerperal septicemia.

Morphologically, the strains of streptococci from smallpox are similar to those isolated from scarlet fever, puerperal septicemia, and erysipelas.

Classification of the streptococci by means of their action on certain carbohydrates would place the organisms from smallpox and those from scarlet fever, erysipelas, and puerperal septicemia, in two distinct groups, based on the inability of the latter to ferment lactose.

The controlling strains each produced an acid reaction in litmus milk while the strains from smallpox were all negative.

The virulence for mice of organisms from smallpox is low, the usual fatal dose, after repeated passage, being about 0.1 c.c.

Agglutination tests with both homologous and heterologous strains would indicate a different serologic grouping between the organisms isolated from smallpox and the comparative strains.

Complement fixation results run about parallel to the agglutination tests and are apparently no more specific.

Streptococci isolated from smallpox produce a specific toxin which when injected into susceptible individuals gives rise to a cutaneous reaction.

The antitoxin produced in rabbits is specific as it neutralizes the toxin while normal serum has no effect.

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CHANGES IN BLOOD CONCENTRATION INCIDENT TO SHOCK*

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MONOGRAPHS on clinical pathology and microscopy contain no references to blood changes in traumatic shock or in circulatory failure of similar character developing in other conditions. Occasional references to such changes are found in clinical reports, but their significance and diagnostic importance have not been emphasized.

In 1893 Sherrington and Copeman[1] observed that animals, subjected to prolonged experimental operative procedures, presently showed increased respiratory and pulse rate, and decreased temperature and blood pressure. This was accompanied by the development of edema and by increased concentration of the blood. They suggested the relationship of these phenomena to surgical shock. It is of interest that these observations forecast by twenty-five years the accepted interpretation of shock, and that the essential features of it including increased blood concentration were noted. Cannon, Fraser and Hooper,[2] observing the development of shock in wounded soldiers, found a red cell count of 6 million or more and a corresponding increase in hemoglobin as prominent characteristics. Robertson and Bock[3] corroborated those observations. Keith[4]

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showed that the increased blood concentration was due to loss of blood plasma and that it was accompanied by a marked decrease in total blood volume. These findings have been confirmed repeatedly. In shock produced experimentally an increased concentration of blood occurred so regularly that we used it as a criterion for shock in a study of the pathology of that condition.[5]

Dale, Laidlaw and Richards,[6] by injections of histamine, reproduced experimentally in mammals all the physiologic manifestations of shock following trauma. They noted increase in the red cell count and hemoglobin as regular features. They found that histamine produces direct injury to capillaries, causing loss of tonus and increasing the permeability. Escape of plasma through damaged capillary walls resulted in concentration of the cellular elements of the blood. The visible circulatory changes produced by histamine are present in cases of traumatic shock and the mechanism of their production apparently is identical. In the final analysis the etiology of shock is loss of capillary tonus. Extensive capillary dilatation withdraws blood from currency as effectively as hemorrhage. The patient "is bled to death into his own capillaries."

Krogh[7] of Denmark and Lewis[8] of England have studied the physiology of the capillaries most extensively and intensively. Their results agree in showing that increased permeability and loss of tonus are produced by all types of damage to capillaries. Such damage may result from light, heat or cold, chemical agents both organic and inorganic, injurious bacterial products and particularly products of cellular injury, similar to histamine in action and in composition. Any of these agents appropriately applied, will produce the shock syndrome. One who, unaccustomed to light, exposes his skin surface to intense sunlight for a short time may develop shock in mild degree. Profound shock has resulted from overexposure to the sun. Similar results may follow overexposure to artificial light rich in ultraviolet rays. It is well known that shock regularly follows superficial burns, and that its degree is proportionate to the size of the burned area. The shock is profound and may be fatal if about one-third of the skin surface is burned or scalded. Many salts of heavy metals will produce shock if introduced into the circulating blood. Various organic compounds will produce similar disturbances. Sodium barbital, and closely related compounds used as sedatives, will produce shock when overdoses are taken. We have seen characteristic circulatory collapse develop in a dog following the intravenous injection of sodium barbital, 0.3 gm. per kg. of body weight. The blood pressure fell 94 mm. in three hours. The respirations and pulse rate became increased to twice the normal. The external parts became cold; there was 20 per cent increased concentration of the blood as shown by hemoglobin percentage and erythrocyte count. At postmortem examination all the changes which we have found to accompany shock were present. These, it should be emphasized, are identical to those observed by Dale, Laidlow and Richards, following death from histamine (see Figs. 1 and 2). The abdominal and thoracic viscera had a deep rose-red color due to capillary congestion. The minute venules everywhere were engorged. The mucosae were dull red in color. There was blood-tinged fluid within the bowel tract and in the serous cavities.

The lungs were irregularly congested and their weight was increased. They contained innumerable small hemorrhages. Capillary hemorrhages were also present in the serous surfaces, the endocardium and in the lining of the gall-bladder and urinary bladder.

A man, aged thirty-four, took an unknown amount of "sedormide." Profound narcosis resulted and he was unconscious for eighteen hours. On admission to the hospital the red cells were 6,030,000 and the hemoglobin 118 per cent. On the following day the temperature was 97°, the pulse 130 and the blood pressure 110/70. There were moist râles throughout the chest and the percussion note was hyperresonant. Adrenalin, atropine, caffeine and pituitrin were given as stimulants. Fluids were given by mouth, by hypodermoclysis and by glucose solution intravenously. These were ineffective. Death occurred within seventy-two hours. The postmortem findings were characteristic of shock. There was general engorgement of all the body viscera. The venules and capillaries of the entire gastrointestinal tract were distended in both the peritoneal and mucosal surfaces. The lungs were extremely dark red, were almost airless and contained quantities of bloody fluid. Petechial hemorrhages were numerous throughout the lungs and in serous surfaces. Excepting the circulatory changes described, and the beginning of bronchopneumonia, microscopic examination showed no significant changes in any organs, including the central nervous system. It is stated[9] that overdoses of sedatives belonging to this group of urea derivatives, cause a marked fall in temperature and of blood pressure due to paralysis of the peripheral vessels. Krogh's experiments[10] with urethane showed that it affected the capillaries producing marked dilatation. He stated that other narcotics produce similar effects. Our microscopic examinations showed marked dilatation of the capillaries and venules in deaths from barbital and from "sedormide." Both the physiologic disturbances and the postmortem findings in such cases are indistinguishable from those of shock.

We have seen identical changes in dogs following the intravenous injection of killed bacterial cultures and following peritonitis experimentally produced. We were able to produce the typical shock syndrome in dogs by the intravenous or intraperitoneal injection of watery extract of normal dog muscle, by introducing muscle substance into the peritoneal cavity or by mechanical trauma to the limbs. In every instance the syndrome was accompanied by increased concentration of blood (Table I). In each instance the visible circulatory changes characteristic of shock were present. These findings support the conclusion that a wide variety of agents are capable of causing capillary damage, and that such damage results in circulatory failure with manifestations resembling traumatic shock.

Circulatory failure of this type, often spoken of as collapse, occurs in various clinical conditions. Toxemia with shock following intestinal obstruction is a common occurrence. It has been attributed to the absorption of histamine from the injured bowel. Underhill[11] noted circulatory collapse following gas poisoning and in influenzal attacks of extreme severity. In each of these instances increased concentration of blood was recorded. Atchley[12] noted the

shock syndrome in diabetic acidosis, in severe infections, and in one instance of a rattlesnake bite in which the venom apparently was injected directly into a vein.

TABLE I

CHANGES IN BLOOD CONCENTRATION IN A DOG. SHOCK WAS PRODUCED BY INTRODUCING SUBSTANCE OF DOG MUSCLE INTO THE PERITONEAL CAVITY. DEATH RESULTED IN 10.5 HOURS

BLOOD CONCENTRATION: SHOCK

TIME	SP. GRAV.	HEMOGLOBIN	RED CELLS
6:30 A.M.	1.060	101	5,110,000
7:00 A.M.	Muscle substance into peritoneum		
10:30 A.M.	1.065	104	4,860,000
1:30 P.M.	1.065	104	5,694,000
3:20 P.M.	1.067	108	5,695,000
4:50 P.M.	1.075	120	6,400,000
5:15 P.M.	1.075	130+	7,450,000

We have had opportunity to make examination following deaths from acute hemorrhagic pancreatitis, mesenteric thrombosis, intestinal obstructions, eclampsia, perforated intestinal ulcer, burns and other conditions in which the shock syndrome was a prominent antemortem feature. In each instance the blood changes and the postmortem findings were those seen in traumatic shock in man, and in experimental shock in dogs.

An experience as pathologist in a large charity hospital during the pandemic of influenza, 1917 to 1919, resulted in observations not interpreted at the time. Many of the most severe cases, which died in from two to four days from the onset, had increased concentration of blood. Red cell counts in such cases ranged from 6,000,000 to 8,000,000 and the hemoglobin was correspondingly high. The attending physicians came to regard this as a grave sign. Those patients with high concentration died early with evidences of circulatory collapse. There was a marked decline in blood pressure, weak pulse, rapid respirations and the temperature was lower than seen in cases less severe. Postmortem examination showed wet bloody lungs and extreme capillary congestion of the mucosae of the pulmonary and gastrointestinal tracts. The patients having moderate concentration of blood lived from seven to fifteen days. On postmortem examination the congestion and edema were not so extreme but were marked. Bronchopneumonia which was very irregular in its characteristics and distribution, was a regular feature. In the most severe cases no pneumonia was demonstrable either grossly or microscopically. The lungs had all the features seen in patients dying within two or three days from severe burns or from physical trauma. They presented no essential differences from the lungs following shock produced experimentally.

A young woman suffering from acute hemorrhagic pancreatitis was brought to the hospital. She had rapid weak pulse, rapid respirations, cold pale skin and falling blood pressure. No operation was done; the treatment was entirely symptomatic; she died of circulatory failure within forty-eight hours. Only one blood count was made. The red cells were 6,400,000 and the hemoglobin 112 per cent. At postmortem the visible changes characteristic of shock were present. The lungs were deeply congested, edematous, and heavy.

Their capillaries and venules were distended with blood, and there were numerous capillary hemorrhages within the lungs, in the pleurae and in other serous and mucous surfaces. The abdominal viscera were diffusely deep red and the minute venules were abnormally prominent. DeTakats and Mackenzie[13] reported 9 cases of hemorrhagic pancreatitis, in all of which the red cells were about five million and the hemoglobin correspondingly high. One case had

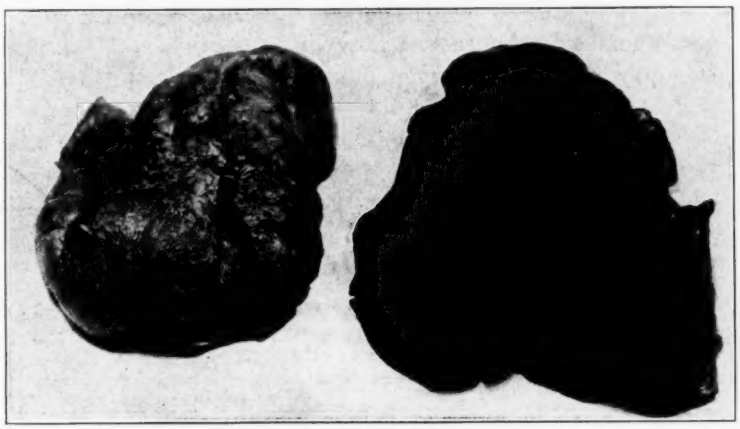

Fig. 1.—Photograph of lung in shock, death within forty-eight hours following severe burn. Normal lung (left) for comparison.

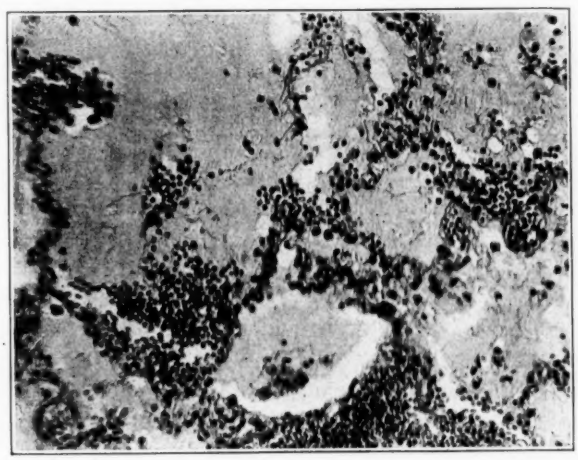

Fig. 2.—Microphotograph of lung, shock following burns. Congestion, edema and capillary hemorrhage are shown.

8,300,000 red cells and 140 per cent hemoglobin. They recognized the shock syndrome in each of these cases and stated that blood counts furnished a means for estimating the degree of shock.

Underhill[14] and his associates have emphasized increased concentration of blood as a clinical feature in burns. They failed to note the relationship of this to the mechanism of shock, but recognized it as a grave physiologic disturbance and applied suitable measures for its relief. The condition is well exemplified in the following cases:

Three men were seriously burned following a collision in which a gasoline tank was burst and ignited. The burns were of the second and third degrees involving chiefly the legs, arms, and faces. Shock was a marked feature in each case. The burns varied somewhat in the amount of skin involved and likewise the degree of shock varied. The man having the most extensive burn had a red cell count of 8,350,000 and the hemoglobin was 120+. The Dare hemoglobinometer which was used had no provision for higher readings. He died within forty-eight hours. The postmortem findings were typically those of shock[5] (Figs. 1 and 2). Another, whose burns were slightly less extensive, died of secondary pneumonia eleven days later. His condition of moderate shock, as shown by blood pressure and concentration, continued during the eleven days. The circulatory changes at postmortem were the same as described. In addition there was diffuse bronchopneumonia in an early stage. This feature is especially significant. Lungs, whose circulation is impaired and whose spaces are filled with edematous fluid, present a stage already set

TABLE II

CHANGES IN BLOOD CONCENTRATION DURING RECOVERY FROM SEVERE BURNS. THE ACCIDENT OCCURRED DEC. 12, 1932; THE FIRST BLOOD EXAMINATION WAS MADE TWO DAYS LATER

BLOOD CONCENTRATION: BURN, RECOVERY

DATE	SP. GRAV.	HEMOGLOBIN	RED CELLS
12-14	1.075	120+	6,700,000
12-15	1.070	112	5,400,000
12-16	1.063	110	5,000,000
12-17	1.070	116	5,200,000
12-18	1.068	115	5,100,000
12-19	1.066	110	4,990 000
12-20	1.062	106	4,900,000
12-21	1.055	99	4,630,000
12-22	1.048	74	3,530,000
12-24	1.045	87	3,820,000

for the final act, the development of pneumonia. The third man who was burned recovered. The blood concentration, shown in Table II, declined gradually as the patient's condition improved.

SUMMARY

Increased concentration of the blood is a regular feature developing during surgical or traumatic shock.

Circulatory failure similar to shock is of frequent occurrence in conditions accompanied by toxemia. Such conditions include burns, toxemias of metabolic origin, poisoning with certain drugs, surgical emergencies such as intestinal obstruction, pancreatitis, and perforated ulcer, and infections of unusual severity.

Increased concentration of the blood, as shown by specific gravity, red cell count and hemoglobin content, is a feature in such cases. The degree of concentration apparently is proportionate to the severity of shock.

The circulatory changes seen at postmortem in such cases are indistinguishable from those of traumatic shock. These observations indicate that the mechanism of origin is essentially the same.

Observations on concentration of blood should be of value in detecting and estimating the degree of shock in clinical studies.

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METAPLASTIC CHANGES IN THE PROSTATE GLAND*

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HISTOLOGIC changes observed in 2 cases of prostatic hypertrophy were of such a character as to lead us to study the behavior of prostatic glandular epithelium.

The first case is that of a man fifty-nine years of age (No. 8,393) who was admitted to the hospital on May 17, 1931. His chief complaint was pain in the left loin and urinary retention of twenty-four hours' duration.

He had been suffering from polyuria, nocturia, and at times dysuria, and also complained of some difficulty in starting the urinary stream and periods of retention. This condition was present for more than five years. There was also loss of weight, digestive impairment, occasional night sweats and blood-streaked sputum.

Examination revealed an elderly undernourished male who appeared acutely ill. Bronchial breathing and a few moist râles were present at right apex and below the right clavicle. A presystolic murmur was heard. The abdomen was distended and presented an ovoid swelling which reached upward to the umbilicus. There was flatness on percussion over this area. Ten-

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derness was present in the left loin. No rigidity was present in the abdomen. The right Murphy sign was positive. Rectal examination revealed a large boggy mass present in the prostatic area with most of the enlargement to the left side. A small rigid catheter was introduced into the bladder with difficulty. Catheterization recovered 52 ounces of residual urine.

A cystoscopic examination showed a marked prostatic hypertrophy especially of the left lobe. There was a generalized cystitis.

White blood cells 16,500, polymorphonuclears 78 per cent, lymphocytes 18 per cent, monocytes 4 per cent, hemoglobin 93 per cent, red blood cells 4,000,000. Urine examination on admission was negative except for a trace of albumin. Blood chemistry showed urea nitrogen 38.5, creatinine 1.5, glucose 110, chloride 460.

On May 19 a suprapubic cystostomy was performed and a mushroom catheter placed in the bladder. The patient had a rather stormy postoperative course because of the myocardial weakness and finally developed pneumonia. He died on June 15.

No consent for a general autopsy was obtained, but we succeeded in removing the bladder and prostate through the surgical incision.

Macroscopically the prostate was considerably enlarged, particularly in its transverse diameter, while the anteroposterior diameter was but slightly increased. The gland was therefore of an elongated oval shape and appeared rather flat. Its size was about twice the normal and felt quite hard to the touch. The central portion showed a superficial irregular defect, the bottom of which was covered with some grayish yellow cheesy material. On cutting through this defect it was found that the grayish yellow discoloration extended irregularly to the depth and seemed to demarcate rather sharply in the form of a saw edged line, from the lateral apparently intact parts of the prostate.

Microscopic Examination.—There was an extensive necrotic area in the center with some leucocytic infiltration of the periphery and some small irregular hemorrhages. In the lateral portion the glands were of the type seen in ordinary prostatic hyperplasia. Their epithelium was high columnar and showed a clear faintly staining cytoplasm. Many glands, however, were lined with a very low cuboidal or flat epithelium and some glands were distended thereby forming cysts, quite a few of which were of considerable size. The amount of stroma was increased throughout, and it was of a fibrous hyaline character. Rather massive fibrous tissue was found in the vicinity of the necrotic focus. The glands which persisted in this scarry area were obviously atrophic, their lumen narrow and often collapsed. Some were transformed into solid epithelial trabeculae. The cytoplasm of these cells was scanty; the nuclei were comparatively large, well stained and round or spindle shaped. There were several solid islands of squamous epithelium between these atrophic glands, some of which showed central parakeratosis while others were almost completely keratinized with the exception of a peripheral seam of cuboidal cells forming a single layer (Fig. 1). In other groups of squamous cells hydropic vacuolization could be visualized. There were also a few small

cell groups in which only the central cells looked like squamous epithelium, whereas the periphery was formed by smaller cells of the basal cell type which showed striking palisading of their nuclei. Finally there were trabeculae consisting of squamous epithelium, scattered in the fibrous tissue and alternating with groups of smaller less differentiated cells. The general appearance of these foci resembled somewhat a scirrhous carcinoma.

Summarizing our findings, we found we were dealing with a glandular hyperplasia of the prostate with interstitial fibrosis and a central focus of anemic infarction, probably due to thrombosis. The fibrotic areas revealed regressive changes of the glandular epithelium, followed by proliferation and metaplastic transformation into squamous epithelium. Parakeratosis was quite conspicuous.

The second case was that of a man aged forty-four years (No. 8,063) who was admitted to the hospital April 20, 1930, complaining of difficulty in urination. The patient's illness dated back about two months when he first noticed nocturia and some hesitancy in starting the urinary stream. About four weeks later pain and burning occurred when he voided.

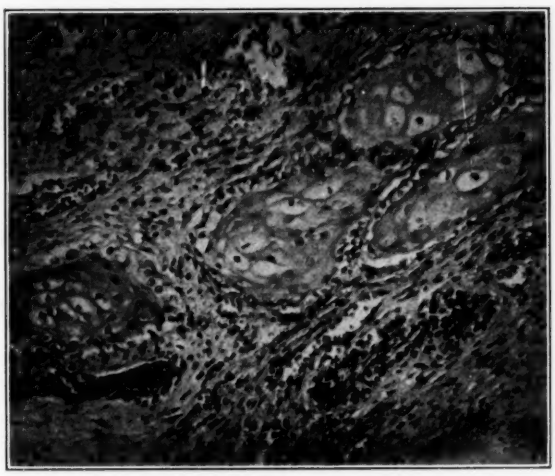

Fig. 1.—Island of squamous epithelium with parakeratosis (high power).

The physical examination was negative except for some tenderness suprapubically. There was a left lumbar scar of a previous nephrectomy. A positive right Murphy sign was present. Examination of the genitalia revealed a hypospadia. On rectal examination a large tender boggy prostate was palpable.

Urine examination showed a trace of albumin and some white blood cells. Blood chemistry showed urea nitrogen 50.3, creatinine 2.9, glucose 143 mg. per 100 c.c.

Cystoscopic examination revealed a marked chronic cystitis with a constriction of the membranous urethra. On pyelogram the right kidney was somewhat enlarged and ptosed. The right ureteral catheter entered the pelvis easily. The left kidney was not present.

On April 23, 1930, a suprapubic cystostomy was performed under spinal anesthesia and a Pezzar catheter sewed into the bladder. Postoperatively the patient's condition improved. This was shown by the decreasing nitrogen retention. On May 9 urea nitrogen was 31.7, creatinine 2.5.

On May 10 a prostatectomy was performed under spinal anesthesia. The patient's condition remained satisfactory until May 13 when he began to hiccough, his abdomen became distended and the temperature began to rise. In spite of the treatment instituted he did not respond well. At this time blood chemistry showed urea nitrogen 44.3, creatinine 5.0, glucose 186. He developed uremic coma with an accompanying hypostatic congestion of the lungs and died May 22, 1930.

The prostate removed was comparatively small, flat and adherent to the surrounding fat tissue. It was very hard and showed a fibrous structure on section with quite a few scattered irregularly formed narrow fissures which occupied mainly the central portion of the more superficial stratum.

Microscopically a fibrosed prostate was found with considerable lymphocytic infiltration, particularly about the very irregularly formed glands. These glands did not resemble the ordinary prostate glands. Some were tubular, others irregularly branching. They were lined with multiple layers of squamous epithelium often causing the lumen to become quite narrow and usually eccentric. Proliferation of the epithelium sometimes closed the lumen altogether and yielded solid epithelial structures. The epithelial cells were usually small, but with quite large nuclei which were round or oval and fairly intensely stained. In other places, particularly in the solid epithelial nests, the cells were smaller, the nuclei densely packed, darker stained, and rod or spindle shaped, thereby resembling basal epithelia.

Careful examination of many slides showed that particularly in the lateral portions a few glands could be found which revealed the ordinary prostatic epithelium with the folding and formation of papillae characteristic of hyperplastic prostatic glands. In some of these glands, transformation of the cuboidal or high columnar epithelium into much smaller cells with darker nuclei could be ascertained. These cells formed several layers and gradually transformed the ordinary appearance of the prostatic gland into such tubular structures as described above.

Summarizing these findings we concluded that we were dealing with a chronic fibrosis of the prostate, apparently of inflammatory nature with transformation of practically all the prostatic glands into tubular structures, lined with squamous epithelium or solid groups of squamous or basal epithelium.

Metaplastic changes of glandular epithelium concomitant with hyperplasia are known to occur in almost any glandular organ. This is the most logical explanation of the observation that glands or mucous membranes covered with columnar epithelium reveal foci of basal or squamous cells. This process was first described in the lungs by Goldzieher who showed that in chronic inflammatory conditions the columnar bronchial epithelium reverts first to cells of the basal type, the proliferation of these cells yielding occasionally considerable thickening of the mucosa and eventually occlusion of the bronchial lumen. The squamous cells form secondarily through differentiation

of the basal cells. This interpretation is based on the conception that the basal cell represents an indifferent type of epithelium to which the columnar cells may revert under unfavorable conditions. The basal cell is considered to be a multipotent cell, which is capable of differentiating into any other type of epithelium.

Goldzieher's explanation of bronchial metaplasia and hyperplasia of the

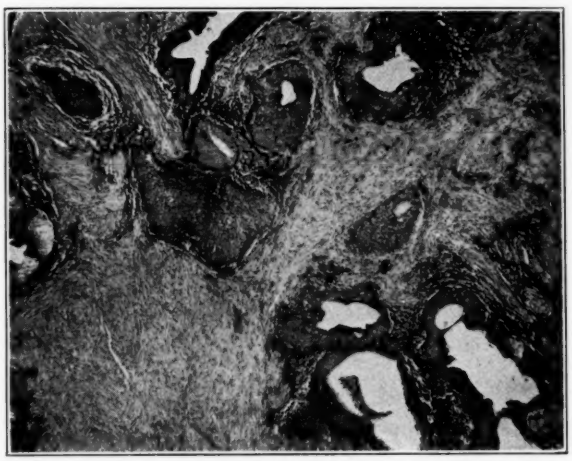

Fig. 2.—Squamous epithelium all along the wall of the prostate glands. In the center a solid island of squamous epithelium (low power).

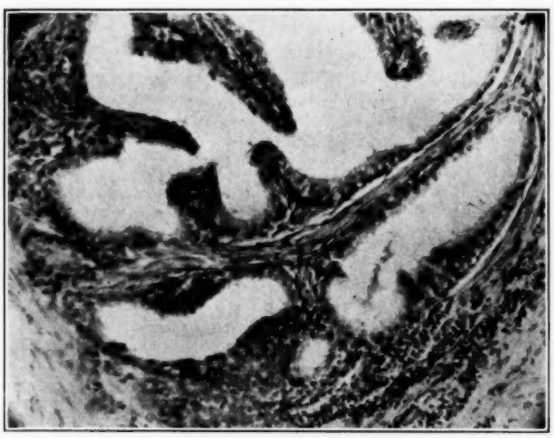

Fig. 3.—Incipient basal cell proliferation substituting the columnar cells of the prostatic gland (high power).

basal cells has been accepted by all the later workers (Teutschlaender, Askanazy, Smith, Fried and others). The experimental studies of Wolbach and Howe have shown, furthermore, that changes similar to those seen in chronic infections can be produced in animals by a diet deficient in vitamins. Basal cell hyperplasia with concomitant metaplasia has been described since in other organs, such as, the pancreas and gallbladder (Krompecher, Roessiger).

The occurrence of solid epithelial nests and of squamous epithelium in the prostate is known since Aschoff demonstrated the physiologic growth of squamous epithelium during the late stage of fetal life. His work was corroborated by Schlachta, who is also of the opinion that remnants of this fetal squamous epithelium may persist in the adult. Schmidt was the first to observe extensive parakeratosis in such islands of squamous epithelium found

Fig. 4.—Advanced basal cell proliferation with beginning differentiation into squamous epithelium (high power).

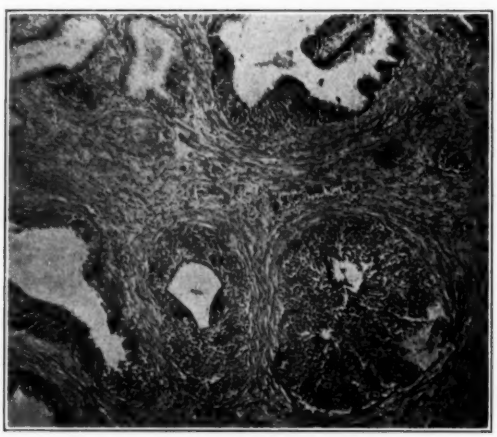

Fig. 5.—Massive proliferation of basal cell, practically obliterating the glandular lumen (low power).

in the adult. He interpreted this lesion in accord with the conception of Schlachta, although these changes were found in close vicinity to an old prostatic abscess. Similar views were held by Tietze and Garfunkel, who observed squamous epithelium in the prostate of eunuchoid and also believed he was dealing with a malformation. Krompecher, however, in a detailed study on the prostatic glandular epithelium showed that there is no need for embryonal nests nor for the hypothetical malformations to explain the histo-

genesis of such squamous epithelium. Their origin from the ordinary glandular cells, or from the epithelium of the prostatic ducts can be clearly demonstrated. The process involved is identical with that which occurs in the bronchial mucosa. According to Krompecher the occurrence of such basal cell hyperplasia and secondary metaplastic differentiation into squamous epithelium is quite common and seems to evolve from regenerative processes in the course of chronic inflammation. In a large series examined for the presence of such epithelial changes, they were observed in 45 per cent of the cases. These figures coincide with our own observation. In reviewing 26 prostates removed during the last eighteen months, 11 cases (that is 44 per cent) show more or less marked hyperplasia with metaplastic changes. Other authors (Grassmann, Neller and Neuburger) give lower figures and estimate the frequency of what they term "atypical epithelial proliferation" at 25 per cent.

There is some difference of opinion as to the actual seat of the lesion. Wagenseil and Altmann believe that the squamous epithelium develops from the ducts of the prostate, whereas, Krompecher found the changes as a rule in the glandular epithelium proper. Our own observations bear out Krompecher's statements, as we frequently observed glands lined partly with ordinary epithelium, while other parts of the same gland already showed definite basal cell hyperplasia. The sections, described in this paper from which the photographs (Figs. 1 to 5) were made, were taken from the lateral portions of the gland so far away from the urethra that there could be no suspicion that the tissue described was urethral glandular tissue. The occurrence of parakeratosis in our first case is the third observation of this kind on record, including those of Schmidt and Altmann.

The changes described in this paper are not only morphologically interesting, but they are also of clinical importance. The presence of squamous epithelium, with or without keratinization and the formation of solid nests and plexi of basal cells in a place where such epithelium does not occur physiologically, may erroneously lead to the assumption of the presence of a neoplastic process. It has been stressed recently by Grassmann that these atypical epithelial formations closely resemble carcinoma, and it is often very difficult to make the differential diagnosis between such lesions and early cancer of the prostate. As a matter of fact there is a frequent coincidence between such changes and carcinoma. This has been noticed by Krompecher, who held the opinion that carcinoma of the prostate, if not of the adenomatous type, is preceded by and develops from such basal cell vegetations. This would explain the occurrence of both basal and squamous cell carcinoma of the prostate. Krompecher's interpretation is based not only on his direct observations in carcinoma of the prostate but also on the analogy with similar observations in other organs, particularly the lungs. A recent paper by Fried also emphasizes the importance of bronchial basal cell hyperplasia for the histogenesis of carcinoma of the lungs. This had already been suggested by Goldzieher.

SUMMARY

Two cases of extensive metaplasia of the prostatic glandular epithelium are described. The histogenesis of metaplasia is discussed in its relationship

to basal cell hyperplasia. The frequency of these changes in the prostate and their significance in the development of prostatic carcinoma is emphasized.

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LABORATORY METHODS

THE DIAGNOSIS OF SURGICAL TUBERCULOSIS*

Relative Reliability of Laboratory Methods

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AT THE New York Orthopaedic Dispensary and Hospital a considerable number of patients are seen with symptoms in a bone, joint, or associated soft tissue which indicate a probable tuberculous lesion. As the pioneer surgery of Dr. Russell A. Hibbs has thoroughly proved, the treatment *par excellence* in such cases is fusion of the affected joint. This presupposes that the diagnosis has been confirmed by methods that are subject to the least possible error.

The ideal method should demonstrate tuberculous infection whenever present and be equally reliable in its negative phase, but as yet no single test has reached this degree of perfection. However, by the judicious combination of several methods, each of considerable accuracy in itself, we believe errors in diagnosis can be reduced to a rarity, indeed, practically excluded.

In demonstration of this contention, we are reporting the results of laboratory examinations made upon all material received from April, 1930, to October, 1932, in which tuberculosis was the object of search. Results are divided into Period A (from April, 1930, to September, 1931, inclusive) and Period B (from October, 1931, to October, 1932, inclusive) since the technic was changed in the latter group. All specimens were examined or confirmed by the author.

Table I

Period A—Tissues (Total 103)

Histology positive—Guinea pig positive	52
Histology positive—Guinea pig negative	7
Histology doubtful—Guinea pig positive	1
Histology doubtful—Guinea pig negative	0
Histology negative—Guinea pig negative	37
Histology negative—Guinea pig positive	1
Histology not supplemented; intercurrent death of pig	5

That is, inoculation confirmed 89 diagnoses, changed 2 to positive and failed to confirm or supplement 12, a percentage reliability of 88.4. Of the

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7 animals which were negative while the corresponding tissue was positive by section, it is most probable that the tissue for inoculation was insufficiently macerated or injected in inadequate amounts, since in three cases subsequent tissue from the same lesion area yielded positive animals. In the tissue which was negative histologically but from which a positive pig was obtained, the material for section came from the head of the femur and that for inoculation from the ilium.

TABLE II

PERIOD A—JOINT FLUID OR PUS (TOTAL 69)

Guinea pig positive	45
Guinea pig negative	19
Guinea pig not supplementary—intercurrent death	5

Percentage reliability in this group was 92.8. Of these 69 specimens, positive animals were obtained in all that revealed acid-fast bacilli by staining. There were 41 which also had tissue sectioned from the same lesion area and the diagnoses agreed in 35, 3 were positive while the fluid or pus was negative, and 3 could not be checked because the pig with corresponding pus died of intercurrent disease.

In summary for Period A, a total of 172 specimens were examined. In 153, animal inoculation confirmed other methods, in 2 it changed a diagnosis to positive, and in 17 it failed to supplement, a percentage reliability for this period of 88.9. Moreover, in at least 3 cases examination of tissue from exploration revealed tuberculosis when the aspirated fluid or pus had been negative.

The factors of intercurrent deaths of some pigs and failure of others to confirm definite histologic evidence of tuberculosis in the above series constituted a serious defect, and in October, 1931, we adopted the technic given at the close of this report, and with the following results:

TABLE III

PERIOD B—TISSUES (TOTAL 57)

Histology positive—Guinea pig positive	33
Histology positive—Guinea pig negative	0
Histology negative—Guinea pig negative	20
Histology doubtful—Guinea pig positive	2
Histology doubtful—Guinea pig negative	1
Histology supplemented—pig died of pyogenic infection	1

That is, inoculation confirmed 54 diagnoses and settled 3 otherwise doubtful diagnoses. Percentage reliability of inoculation in this group was 100. In each of the 2 tissues doubtful by section both animals died of tuberculosis and in the one tissue doubtful by section both animals were negative. In the tissue classed as supplemented, both animals died (four and six days after inoculation) of a staphylococcus peritonitis, the tissue by section was a definite pyogenic inflammation and culture of tissue yielded staphylococci. In 4 other specimens one animal died of intercurrent disease but its duplicate subsequently died of tuberculosis or was negative by autopsy after sixty days.

TABLE IV

PERIOD B—JOINT FLUID OR PUS (TOTAL 28)

Guinea pig positive	18
Guinea pig negative	10
Guinea pig nonconclusive	0

Percentage reliability of inoculation in this group was 100. All material in which acid-fast bacilli were found yielded positive pigs. In 17 cases tissue from the same lesion area was examined and the diagnosis agreed with the findings from fluid. Also in this group 4 animals died of intercurrent disease but the duplicate remained to complete the diagnosis.

During the interval covered by both periods there were also 8 spinal fluids inoculated into animals with no intercurrent deaths; and 18 urine sediments inoculated with one death from intercurrent disease in period A. This last animal, however, did not develop pneumonia until the fifty-fourth day after inoculation and at autopsy its spleen, liver and lymph nodes were negative, so that it may properly be considered as negative for tuberculosis.

From the results in Period B we therefore feel that tuberculosis of the bones, joints and associated soft tissues can be diagnosed or excluded with great reliability if the following methods of examining material are employed:

Tissue.—In the absence of conclusive evidence from pus, fluid, etc., and with the physical findings indicative of tuberculosis, exploration is performed and several small portions of suspected tissue removed. A frozen section of a portion is made at once and, if diagnosed positive by an experienced observer, any therapeutic surgery that may be indicated can be proceeded with. In such cases completion of tissue section by paraffin or celloidin is the only confirmation needed. Without frozen section or when it is not clearly positive, a portion of tissue is expeditiously sectioned by celloidin or paraffin and, pending its completion, a similar portion prepared as follows:

Place tissue in sterile glass mortar, add from 5 to 8 c.c. of sterile saline and a small amount of sterile emery (medium) powder, and macerate thoroughly; add an equal amount of 6 per cent sulphuric acid, mix and place in 37° incubator for thirty minutes; transfer all but coarse fibers and emery sediment to centrifuge tube with a pipette and run at high speed (from 2,500 to 3,000 r.p.m.) for thirty minutes; remove supernatant fluid, resuspend sediment in 6 c.c. of sterile saline and set aside in ice box.

Sections should be completed within three to four days and, if cytology is clearly tuberculous, no further confirmation is needed and prepared sediment in ice box is discarded. When sections are negative or doubtful, proceed with animal inoculation of prepared sediment as follows:

Use guinea pigs of from 250 to 300 gm. that have been quarantined for three weeks following receipt in laboratory. For each sediment suspension inoculate two pigs both intraperitoneally, giving one 2 cc.. and the other 4 c.c. Each animal is housed in a separate cage and observed daily. Inoculated pig is killed when appreciably ill or at the end of sixty days. When autopsied by an experienced observer, typical gross tuberculous lesions of spleen, liver, etc., are considered conclusive; otherwise, or when gross lesions are doubtful, acid-fast stains on smears made from involved viscera shall be the final criterion.

Pus.—Routine cultures and smears for Gram's and acid-fast staining are made. The remainder, up to 5 c.c., is treated with an equal amount of 6 per cent sulphuric acid, etc., as under preparation of tissue sediment, and the resuspended sediment inoculated into two animals in like amounts.

Joint Fluid.—Immediately on withdrawal mix with several cubic centimeters of sterile sodium citrate (2½ per cent) solution to prevent coagulation and then proceed as with pus.

Spinal Fluid.—Unless distinctly turbid or pyogenic organisms found in smears, inoculate directly into two animals, using 3 c.c. and 6 c.c. respectively. If turbid, or pyogenic organisms are present, treat a 10 c.c. portion with sulphuric acid, etc., as under pus, resuspend sediment in 6 c.c. of saline and inoculate in amounts of 2 c.c. and 4 c.c.

SUMMARY

Laboratory methods for the diagnosis of surgical tuberculosis and results of their use are given which, the author believes, warrant the following conclusions:

1. By these methods tuberculosis can, with rare exceptions, be definitely diagnosed or excluded.

2. Negative results by any or all of these methods in the case of joint fluids or pus do not completely exclude tuberculosis. In such cases, where the physical findings, etc., remain suspicious, exploration and examination of tissue are indicated.

513 Main Street.

IMPROVED DRINKING FOUNTAIN FOR SMALL LABORATORY ANIMALS*

Douglas P. Murphy, M.D., Philadelphia, Pa.

IT IS convenient to attach the water reservoirs to the outside of the doors when cages are stacked closely, and one upon the other. The inverted bottle type of drinking fountain is inexpensive and lends itself readily to this purpose.

Large bottles require simple curved or angulated glass nipples. Bottles of not more than 100 c.c. capacity are required for cages with doors only 8 inches high, in order that they will not prevent the opening of the door of the cage immediately above. The partial vacuum created by the inversion of the small bottle using simple curved or angulated nipples may impede greatly, or prevent entirely, the flow of water. The nipple shown in Fig. 1 was devised and has proved satisfactory in overcoming this difficulty.

It is made of glass tubing 9 mm. in outside diameter, and is blown in the form of a gentle curve instead of with an angulation. The lower part is

*From the Gynecean Hospital Institute of Gynecologic Research, School of Medicine, University of Pennsylvania.

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tapered to approximately one-half of its original diameter, and the open end is pressed into the shape of an hourglass.

The hole at the middle of the nipple is its most important feature. As the animal drinks by licking the end of the nipple, bubbles of air form at the hole in the midpoint of the nipple, enter, glide easily around the curve and rise to the surface of the water where they tend to equalize the partial vacuum. As a result, the animal has no difficulty in drinking.

The curve of the nipple facilitates the uninterrupted movement of the bubbles of air.

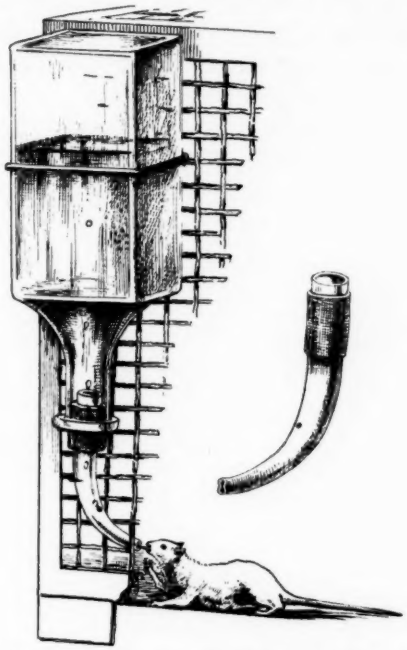

Fig. 1.—Showing construction and mode of operation of glass nipple suitable for 100 c.c. inverted bottle type of drinking fountain, attached to the outside of cage door. Note espcially the 1 mm. diameter hole in the nipple at the midpoint of the curve and upon its concave aspect; also that the bubbles of air form at this point as the animal drinks.

The position and size of the hole, however, are the most important considerations in the construction of the nipple. The best site for the hole is on the concave aspect of the curve at its midpoint. Its optimum diameter is 1 mm. If the hole is smaller, the bubbles form with great difficulty if at all. If the hole is larger than 1 mm., they form more readily, are larger and more numerous, but when the animal stops drinking, they continue to form and the bottle empties automatically.

The credit for developing the nipple belongs to Charles W. Miller, glass blower, The Laboratories, Philadelphia General Hospital.

A SIMPLE AND EFFICIENT REFLUX CONDENSER*

A. BERNHARD, NEW YORK, N. Y.

IN THE determination of cholesterol in blood serum and plasma, reported in a previous communication,[1] it was found that the test tube condenser utilized was subject to certain mechanical difficulties. The water passing through the condenser had to be carefully regulated so that the pressure would not be too great; also, a slow boiling water-bath was essential to prevent condensation of steam into the Folin tube with subsequent interference with the color formation.

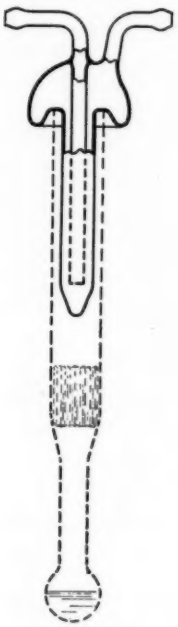

Fig. 1.

All defects were obviated by the use of the condenser shown in Fig. 1. The entire condenser is a single unit of glass, the top of which is made mushroom shape.

The condenser can be used with a vigorously boiling steam bath, and with a greater rate of flow of the condenser water, thereby increasing the efficiency of the condenser.

The particular shape of the condenser and the manner in which it fits the top of the Folin tube prevent any condensation of steam on the inside of the Folin tube.

The condenser was made by Eimer and Amend, New York, N. Y.

REFERENCE

1. Bernhard, A., and Drekter, I. J.: A Simple and Accurate Method for the Determination of Cholesterol in Blood Serum and Plasma, J. LAB. & CLIN. MED. 16: 1225, 1931.

*From the Achelis Laboratory, Lenox Hill Hospital.
Received for publication, February 11, 1933.

CYTOLOGIC STUDY OF BODY FLUIDS*

A. A. Eisenberg, M.D., Harry Wallerstein, M.D., and Aaron Plato, B.Sc.,
New York, N. Y.

AN INFREQUENTLY practiced but highly valuable aid to diagnosis has reappeared in the literature following a lapse of several years. It is not at all uncommon for the medical practitioner to encounter a patient with a seemingly unaccountable effusion in one of the body cavities. That mechanical and metabolic factors, nonneoplastic in nature, may be the cause of such an effusion is a well-established fact. It is also known that certain inflammations, bacterial, chemical, or otherwise, may produce this fluid. It becomes the problem of the practitioner, in such an instance, to determine whether the effusion is due to one of the above factors, or whether it is produced through the mechanism of a neoplasm of the serous membrane or its circulatory supply. Roentgenologic study in these cases is usually of little value unless the fluid is removed before radiography. Even then, the uncertain data gathered from the x-ray (as to the nature of the dyscrasia, whether inflammatory or neoplastic, and if neoplastic, whether primary or secondary) can be transformed into certain knowledge by a study of the cells found in that fluid. For these occasions, it has become possible, in recent years, to offer a valuable and quite accurate aid to the diagnosis, in the rôle of a cytologic study of the fluid sediment.

Since the publication of Mandlebaum's technic for cytodiagnosis of serous fluids appeared in 1917, it has been possible, with a considerable degree of accuracy, to rule out cardiac, inflammatory and metabolic disturbances, to determine whether a given serous effusion has a neoplasm as its source.

Mandlebaum's achievement followed upon the investigations of many workers who slowly, and step by step, prepared the field for the modern-day methods of single tissue cell diagnosis. Attempts to identify tumor cells in body fluids date back to 1860 when Beale[2] first noticed large multinucleated cells in unstained smears of sputum in a case of carcinoma of the pharynx. Little attention was paid to this, as well as to the work of Quincke[6] and of Ehrlich,[3] each of whom in 1882 demonstrated with new staining methods the presence of tumor cells in smears of pleural and peritoneal effusions. The first attempts at using cut sections were made in 1895 by Bahrenberg[1] who allowed a large quantity of fluid to clot spontaneously and then hardened the clot in alcohol, imbedded it in celloidin and cut it as a tissue. In 1900, Mandlebaum[4] demonstrated the presence of actinomyces in pus, by means of tissue-like sections, and it is this method which he described in 1917[5] as being of value when seeking tumor cells in fluids, and which has become the foundation upon which all later methods were based.

*From the Pathological Laboratories, Sydenham Hospital.
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Mandlebaum's method, as practiced at Mount Sinai Hospital in New York was the basis for a very exhaustive report in 1928 by Zemansky,[7] who evaluated 914 examinations made at that institution over a period of fifteen years. His conclusions show that this study can be of inestimable value in arriving at a diagnosis. He emphasized also the worth of a positive report, stating that a negative report was not as conclusive, since the cells could be destroyed or missed even when present. His positive results, however, he regarded as being almost pathognomonic, since in the presence of a sheet of epithelial cells or of only a single cell with mitotic figures, an accepted diagnosis of neoplasm could be made. His checked results bore out these conclusions. At this point it may be advisable from our experience to add a cautioning statement: occasionally a sheet of endothelial cells may be present, having desquamated from the serous membrane. This may closely resemble squamous epithelium or even the medullary cells in a solid carcinoma (Fig. 1).

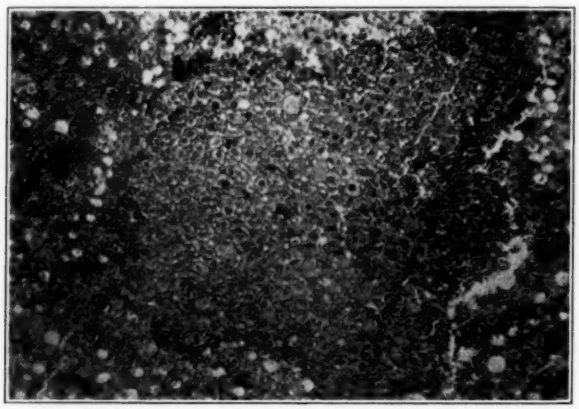

Fig 1.—Note sheet of large, pale, peritoneal cells. These are not neoplastic.

In his large series of cases Zemansky was able to arrive at an accuracy of 90 per cent in his positive results as compared to an accuracy of only 47 per cent in his negative results.

In the much shorter series that we have to report, we have been more fortunate in gaining a somewhat higher percentage of correct results. Over a period of five years, we have had 26 patients on whom we have made 39 examinations. Of these there were 32 positive diagnoses, with one case disproved (Case 25), for an accuracy percentage of 96.9 per cent. In only 7 cases were negative diagnoses made, and these were all checked as nonneoplastic, for an accuracy percentage of 100 per cent. From these statistics it can be seen that in all our cases where tumor cells could have been expected to be present they were found at the microscope. In no positive case was the diagnosis missed, as all our negative results were checked as such by the subsequent history of the case.

Though our series is too short to make the accuracy percentages definitely acceptable, we feel that our results have been so good as to justify the describing of our method, which is a modification of Mandlebaum's, and which we be-

lieve more readily preserves the cells, thus increasing the number of positive diagnoses, decreasing the number of negative diagnoses, and giving a greater general accuracy in cytologic study by this method.

Technic.—The original technic as described by Mandlebaum consisted in preparing the fluid sediment for cutting as a tissue section. This he did by centrifuging the fluid in large conical tubes and getting a sediment which he hardened in 10 per cent formalin and then ran through the alcohols, through chloroform and into paraffin. The hardened block of sediment was then cut, stained and mounted as a tissue section.

The accuracy of the positive results is well attested to by Zemansky's experiences, but the negative results are painfully inaccurate. The reason for this is obvious. In any test where the result depends on the finding of a few isolated cells, a number of factors may enter to cloud the picture. It is possible, for instance, to have a neoplasm involving the pleura with a consequent effusion, and yet have no tumor cells in that fluid. It is possible that no cells have desquamated. It is also possible that so few cells have desquamated that they are extremely difficult to find. For that reason Mandlebaum cautioned that as much fluid as is possible should be obtained and centrifuged. Another possibility is that the tumor cells, subjected to a prolonged sojourn in an environment to which they are certainly not adapted, may be changed to a considerable extent, and that the strenuous regime of fixation, dehydration, clearing, and infiltration to which they are subjected, may so further distort them as to make them unrecognizable, or that these processes may destroy the cells completely. It is true that most frequently the cells remain recognizable in sufficient numbers to permit a correct diagnosis. But, as Zemansky stated, it often happens that one gains an impression that there is a malignancy present, while still unable to give a diagnosis, and also one occasionally gives a negative diagnosis, only to find on subsequent examination or in the future course of the case that a neoplasm was present.

It is for these reasons that we have slightly modified Mandlebaum's technic, seeking to produce less strain on the chemical and physical properties of the neoplastic cells, and thus more often preserving them for diagnosis. The method employed is as follows:

1. Obtain sediment by centrifuging the fluid in large conical tubes at from 1,200 to 1,700 revolutions per minute for from one-half to one hour. Wash sediment several times in normal saline solution.

2. Decant supernatant fluid. Replace with about 25 times the volume of 5 per cent formol acetic acid, at 37° C. for from four to six hours.

3. Dehydrate by steeping through the following solutions:

a. 75 per cent alcohol for one hour

b. 95 per cent alcohol for one hour

c. 2 parts absolute alcohol and 3 parts ether for one-half hour

d. ether for one-half hour

e. 2 parts ether and 3 parts acetone for one-half hour

f. acetone for one-half hour

4. Clear in choloroform.

5. Infiltrate with paraffin at 45° C. gradually increasing the temperature so that in two hours it reaches 56° C.

6. Embed in paraffin as with ordinary tissue blocks.

7. Cut with sharp knife at slight angle, thus exposing a greater surface of the sediment for staining.

8. Staining and mounting is carried out as for routine tissue work.

9. Cut many slides to decrease chance of missing cells.

Comment.—1. Five per cent formol acetic acid is used as a fixative in place of 10 per cent formalin because the latter fixes the cells too rapidly and may produce considerable distortion, while the former permeates through the cells more slowly without injuring their structure.

2. The sediment is dehydrated more slowly and in a graded manner, again avoiding rapid, distorting changes in their appearance.

3. Cutting the block with the knife edge at a slight angle exposes a greater surface of cells and nuclei for staining.

CASE REPORTS

CASE 1.—Mrs. R. D., a white female aged forty-eight years, was admitted to Sydenham Hospital on Oct. 7, 1932, with symptoms of an enlarging abdomen for some weeks and

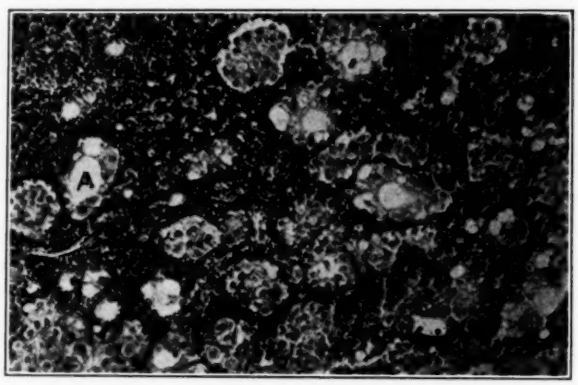

Fig. 2.—Authors' Case 1. Abdominal fluid. Diffuse peritoneal carcinomatosis. *A*. Note acinus formation.

with urinary disturbances. A roentgenologic series was of no aid. There were 8,000 c.c. of straw-colored fluid in the abdomen, which on examination by the above method showed adenocarcinoma (Fig. 2). Laparotomy revealed large thick, white patches on the parietal peritoneum and between the uterus and urinary bladder. The cecum was immovable. Microscopically these patches showed diffuse carcinomatosis.

CASE 2.—Mrs. J. G., a white female aged sixty-nine years, was admitted to the Sydenham Hospital on Aug. 23, 1932, with a pleural effusion on the right side. A chest tap was productive of 1,600 c.c. of light amber fluid, which showed many small clumps of dark staining epithelial cells. A diagnosis of metastatic carcinoma was made, and again at two subsequent tappings, at which occasions bloody fluid was removed. During this time it was learned that four years previously the patient had had a left-sided mastectomy for carcinoma.

CASE 3.—Mrs. V. M., a white female aged twenty-nine years, was admitted to the Sydenham Hospital on June 2, 1932, with the diagnosis of serous pleurisy, possibly tuberculous. Examination of the fluid showed the absence of tumor cells, and the presence of an exudate with a large preponderance of lymphocytes and some hyaline material resembling

caseation. No giant cells were seen. A diagnosis of probable tuberculosis was made, and confirmed by the result of guinea pig inoculation.

CASE 4.—Mrs. B. B., a white female aged forty-three years, was admitted to the Sydenham Hospital on Nov. 16, 1931, with fluid in the right chest, cough and pain on that side. A tap was productive of 750 c.c. of sanguinous fluid which showed on cytologic study large groups of neoplastic cells of the kind frequently seen in tumors of the ovary. Six subse-

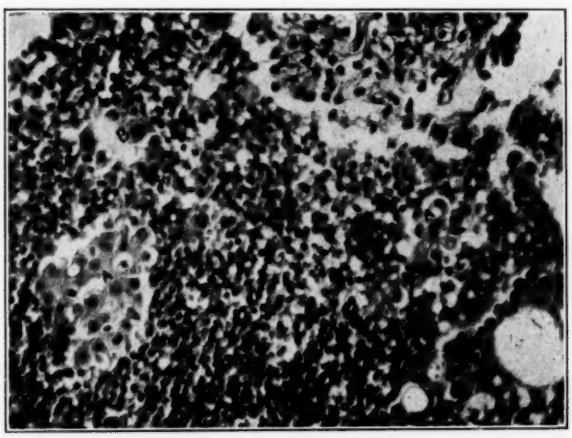

Fig. 3.—Authors' Case 4. Chest fluid. *A*, Note mitoses; *B*, double nuclei.

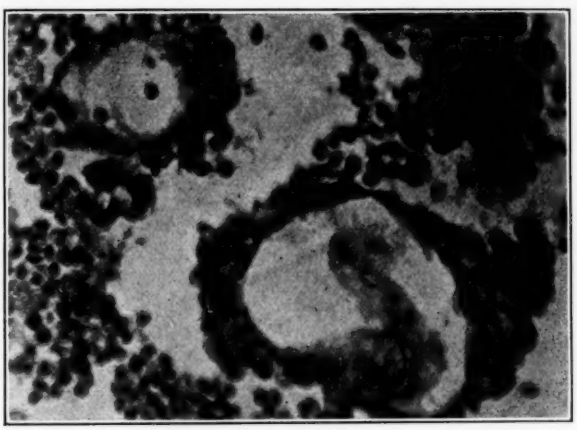

Fig. 4.—Same case as Fig. 3. Chest fluid. Note close resemblance to cells in ovarian carcinoma.

quent thoracenteses resulted in the same findings and gynecologic examination disclosed a large mass in the left adnexal region. Operation was performed and both ovaries removed. Microscopically there was a bilateral papillary adenocarcinoma of the ovaries Figs. 3, 4, and 5.

CASE 5.—Mrs. R. N., a white female aged thirty-four years, was admitted to the Sydenham Hospital on Feb. 3, 1932, with a cough and a left-sided pleural effusion. Aspiration resulted in a sanguinous fluid that showed a denocarcinoma on microscopic study (Fig. 6). The roentgenologic diagnosis after the tap was metastatic involvement of the lungs secondary to pleural malignancy.

CASE 6.—Mrs. B. F., a white female aged fifty-six years, was admitted to the Sydenham Hospital on Feb. 15, 1932, with pain in the chest, dyspnea even at rest, and paroxysms of coughing. There was no hemoptysis, and no signs of congestive circulatory failure.

Thoracentesis was productive of a hemorrhagic fluid which showed a probable pleural endothelioma. This was confirmed by x-ray, and later by necropsy at another institution (Fig. 7).

CASE 7.—Mrs. H. B., a white female aged eighty-three years, was admitted to the Sydenham Hospital on Dec. 21, 1931, with a cystic mass the size of an orange in the right kidney region. It was found to be a cyst in the sacroiliac joint. The aspirated fluid showed an epithelial malignancy. Other metastatic foci were found at radiographic study, but the primary tumor was not located.

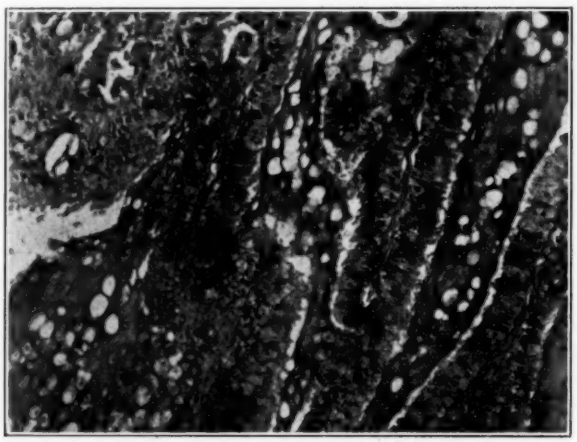

Fig. 5.—Papillary adenocarcinoma (bilateral). Same case as Fig. 3. Ovarian tumor. *A*, Note resemblance to cells in chest fluid.

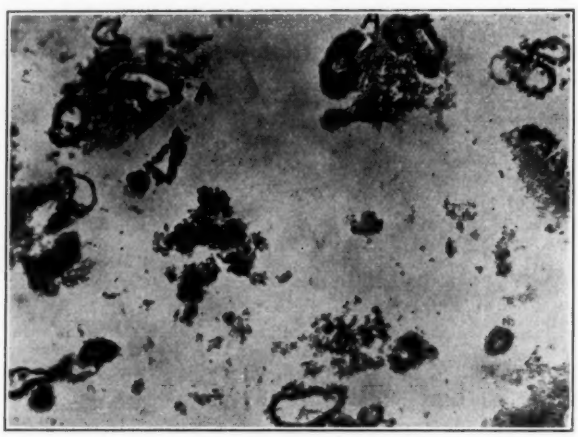

Fig. 6.—Chest fluid in case of breast carcinoma. *A*, Note ductal formation and resemblance to breast tumors.

CASE 8.—Mrs. P. K., a white female aged thirty-eight years, was admitted to the Sydenham Hospital on March 20, 1930, for parturition, with cough and pain in upper right chest. Two years previously she had had a right mastectomy for carcinoma. Roentgenologic study showed fluid in the right pleural cavity. At thoracentesis a hemorrhagic fluid was found, which on section of the sediment with microscopic study thereof showed sheets of neoplastic epithelial cells. The patient later died at another institution.

CASE 9.—Mr. M. L., a white male aged forty-two years, was admitted to the Sydenham Hospital on April 25, 1932, with generalized icterus, loss of weight, weakness, and a vesicular

rash on his lower extremities. The axillary and inguinal lymph nodes were considerably enlarged. There was a marked pleural effusion which was tapped on two occasions, and each time showed the presence of many eosinophiles, reticulum cells, endothelial cells, and a suggestion of Dorothy-Reed cells. The diagnosis of Hodgkin's disease was made, and confirmed by the subsequent course of the disease, although no biopsy was made. The patient is under a deep x-ray therapy and follows a typical Hodgkin's disease course.

CASE 10.—Mr. J. S., a white male aged forty-three years, was admitted to the Sydenham Hospital with a history of sudden ascites that came on a few weeks previously. At

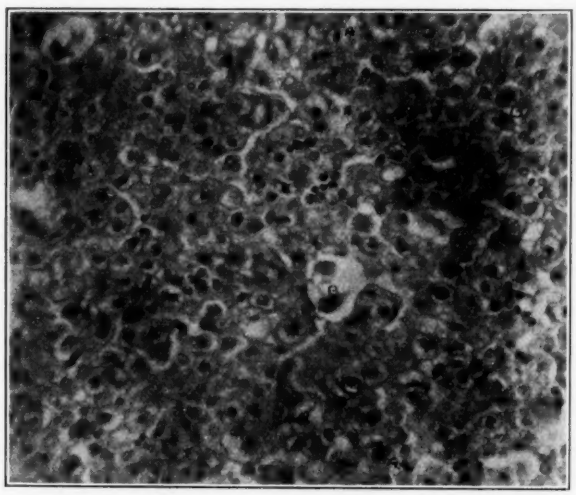

Fig. 7.—Authors' Case 6. Chest fluid. Note typical foamy cells with vesicular nuclei. *A*, Mitoses; *B*, double nuclei.

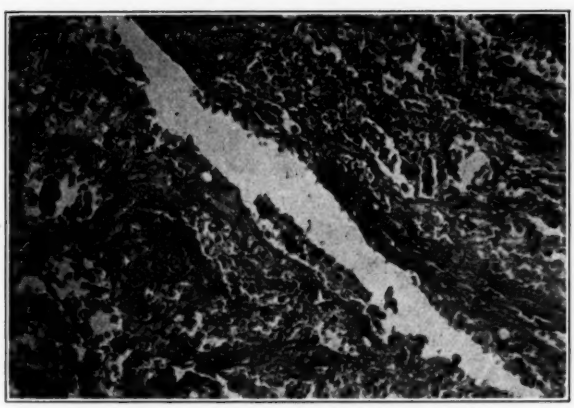

Fig. 8.—Authors' Case 8. Abdominal fluid. Note diffuse carcinomatosis. Scirrhous type arrangement.

laparotomy there were 10 quarts of fluid and a diffuse peritoneal carcinomatosis. Examination of the fluid sediment showed numerous large spheroidal epithelial cells with acidophilic cytoplasm and pyknotic nuclei (Fig. 8). The primary focus was not located.

CASE 11.—Miss M. H., a white female aged thirty years, was admitted to the Sydenham Hospital on May 3, 1929, with vague abdominal pains and ascites, associated with loss of weight and strength. The abdominal fluid was aspirated and showed numerous epithelial cells of various sizes and shapes with numerous mitotic figures. There were many large

cells, hydropic with the nuclei displaced to the periphery giving the typical "signet ring" cells (Fig. 9). The diagnosis was confirmed at operation.

CASE 12.—Mrs. B. H., a white female aged sixty years, was admitted to the Sydenham Hospital on July 3, 1929, with a history of diabetes, complaining of dyspnea, and having fluid in the chest. Thoracentesis resulted in a hemorrhagic fluid which showed microscopically many epithelial cells piled up on a fibrinous basement membrane in papillary arrangement. A diagnosis of papillary carcinoma was made, but was not substantiated since the patient left after the fluid recurred, and has not been heard from.

CASE 13.—Dr. S. H., a white female aged forty-seven years, was admitted to the Sydenham Hospital on Nov. 6, 1930, with dyspnea, cough, abdominal distention, and vague pains in the abdomen and chest. Radiograph disclosed the presence of large abdominal glands, and fluid in the chest. Hemorrhagic fluid was removed which showed many pus cells and numerous reticulum cells and eosinophiles with endothelial cells of the type seen in Hodgkin's disease. The diagnosis was confirmed by biopsy.

CASE 14.—Mr. J. S., a white male aged thirty-six years, was admitted to the Sydenham Hospital on Jan. 27, 1932, with cough, expectoration of tenacious sputum (often blood-tinged)

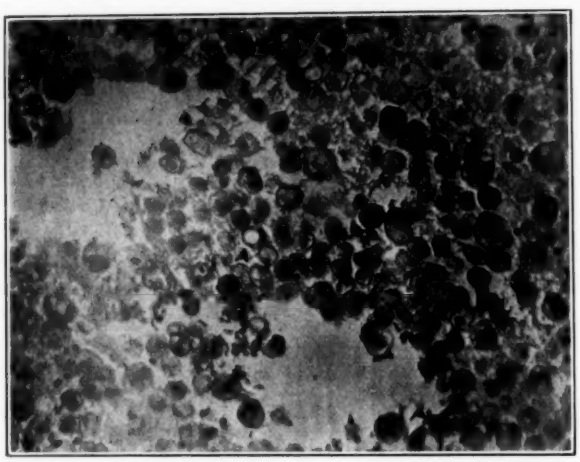

Fig. 9.—Authors' Case 11. Abdominal fluid. *A*, Note typical "signet ring" cells as seen in Krukenberg tumor of ovary.

and dyspnea with pleuritic pain on both sides of the chest. At thoracentesis a hemorrhagic fluid was aspirated which showed many sheets of epithelial cells, many with mitotic figures. A diagnosis of epithelial malignancy was made, and in March, two months later, bone metastases were found. The patient died shortly afterward, and since no autopsy was performed the primary focus was never ascertained.

CASE 15.—Mrs. C. C., a white female aged thirty-seven years, was admitted to the Sydenham Hospital on April 7, 1932, with a postpneumonic lung abscess, and fluid in the right chest. Aspiration was productive of a thick yellow fluid which showed no tumor cells, and many leucocytes. A negative diagnosis was made, and the patient subsequently recovered.

CASE 16.—Mrs. B. H., a white female aged fifty-eight years, was admitted to the Sydenham Hospital on Dec. 5, 1932, with precordial distress, weakness and loss of weight (50 pounds in two years). Her sputum was mucoid and blood-streaked. Large nodes were felt in the region of the bifurcation of the carotid (right). Aspiration of the fluid in the right pleural cavity produced brownish yellow fluid which showed on microscopic examination numerous spheroidal epithelial cells with round vesicular nuclei and many mitotic figures. A diagnosis of epithelial malignancy was made, and confirmed by x-ray, but the primary focus was never found.

CASE 17.—Dr. A. W., a white male aged fifty-two years with loss of weight and painless jaundice for six months. He had had a cholecystectomy two years previously, and prior to that had had a finger amputated because of an epithelioma which had developed due to his work with x-rays. The growth never recurred. Shortly before his death he developed an ascites, which was aspirated and showed a clear amber fluid with many spheroidal epithelial cells with irregular nuclei and mitotic figures. A clinical diagnosis of carcinoma of the pancreas was made, but no further substantiating evidence was forthcoming.

CASE 18.—Mrs. T. S., a white female aged eighty-five years, with urinary disturbances, frequency, dysuria. Her bladder was washed and the washings on two separate occasions examined as for cells in an effusion. The microscopic study showed many inflammatory cells and many sheets of papillomatous formations, that showed malignant changes in the cells. A diagnosis of papilloma with malignant degeneration was made and confirmed by the cystoscopic examination.

CASE 19.—Mr. A. L., a white male aged fifty-three years, was admitted to the Sydenham Hospital on April 4, 1931, with complaint of pain in the right chest. Several tappings were productive of straw-colored fluid which failed to show any neoplastic cells, but did contain many lymphocytes and monocytes. Radiography disclosed a large mass in the middle lobe of the right lung, and a diagnosis of pulmonary neoplasm was made notwithstanding the negative reports from the laboratory and the presence of a positive Wassermann test. At autopsy a large gumma of the lung was found.

CASE 20.—Mr. S., a white middle-aged man was found by his physician to have fluid in the pleural cavity. The physician aspirated this fluid, which was hemorrhagic in nature, and brought it to the laboratory for cytologic study. Paraffin sections of the sediment showed many plasma cells and many large, foamy cells, probably endothelial in origin with double nuclei and mitotic figures. A diagnosis of probable pleural endothelioma was made, but was not confirmed as the patient was lost track of.

CASE 21.—Baby T., a white male aged three and one-half years, was brought to his physician in a state of suffocation. The doctor suspected an enlarged thymus, had the child studied radiographically, and found fluid in the pleural cavities with diffuse nodular growths in the lungs. Study of the fluid sediment showed many lymphocytes and monocytes with many reticulum cells. A diagnosis of mediastinal lymphosarcoma was made and confirmed by the x-rays and subsequent course of the disease. The child was improving remarkably with deep therapy, but finally succumbed after two years.

CASE 22.—Mrs. W., a white female aged sixty years, was treated by her physician for fluid in the chest. She had rapid refilling after each tapping. The fluid was hemorrhagic in nature and microscopically showed many sheets of palisade-arranged cells which were uniformly round and oval, foamy with vesicular nuclei. A diagnosis of pleural endothelioma was made and confirmed by autopsy later.

CASE 23.—Mrs. E. W., a white female aged fifty-eight years, was admitted to the Sydenham Hospital on Jan. 10, 1933, with abdominal ascites. She had been laparotomized at another hospital several months previously, and was admitted to this institution for paracentesis. A paracentesis resulted in 1,800 c.c. of straw-colored fluid which showed numerous epithelial cells in solid clumps and in acinous formation. Many mitotic figures were seen. A diagnosis of epithelial malignancy was made and confirmed by the previous laparotomy.

CASE 24.—Mrs. A. M., a white female aged sixty-two years, was admitted to the Sydenham Hospital on Sept. 26, 1932, with marked anemia, some jaundice and a history of loss of twenty-five pounds over a period of three months. A calculus was found in the left kidney and also a lumbar arthritis was discovered. There was an abdominal ascites which at paracentesis yielded a straw-colored fluid. Microscopic study failed to reveal any neoplastic cells. At operation there was a chronic cholecystitis with stones in the cystic and common ducts. She proceeded to make an uneventful recovery.

CASE 25.—Mrs. D. G., a white female aged thirty-seven years, was admitted to the Sydenham Hospital on June 9, 1931, for hysterectomy for a fibroid uterus. She had an uneventful recovery and was discharged, but returned on Dec. 8, 1932, with ascites and a rup-

tured duodenal ulcer. The abdominal fluid was examined microscopically and a diagnosis of an epithelial malignancy was made, but not confirmed at operation.

CASE 26.—Mrs. M. D., a white female aged forty-one years, was admitted to the Sydenham Hospital on Jan. 27, 1933, with a history of illness for four months, her symptoms simulating an acute upper respiratory infection. Her condition became progressively worse and the loss of weight became marked. She developed a large amount of fluid in the right pleural cavity. A thoracentesis was productive of 600 c.c. of bloody fluid, and three days later 400 c.c. more were removed. Microscopic study of the sediment showed an epithelial neoplasm. The radiographs gave a picture of primary growth of the lower lobe of the right lung.

SUMMARY

1. There is presented a modification of the present-day methods of utilizing the sediment in exudates as tissues for histologic study. This modification causes less stress on the chemical and physical properties of the cells, thus preventing their distortion and preserving them for diagnosis.

2. Twenty-five cases, carefully checked up by subsequent histories, operations, and autopsies whenever possible are added to the literature.

3. The authors feel that the study of fluid sediments from a histopathologic viewpoint can prove a very valuable adjunct to clinical diagnosis, in addition to pointing the way for therapy in doubtful cases.

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565 MANHATTAN AVENUE.

SIMPLE METHOD OF DRYING PIPETTES*

VIRGINIA M. WALKER, MOBERLY, MO.

A SIMPLE and inexpensive device for drying pipettes, where a laboratory does not have access to compressed air, may be made by using an ordinary air pump for automobile tires.

This pump may be fastened securely in an upright position on the worktable near the sink. The rubber tubing is placed over the end of the pipette, and two or three pressures of air will be sufficient to dry any pipette of ordinary size.

If one has a properly fitting adapter for a Luer type syringe, it may be placed on the end of the rubber tubing and hypodermic needles dried in the same way.

*From the Woodland Hospital.
Received for publication, April 19, 1933.

THE PURIFICATION OF GUM DAMMAR FOR USE AS A MOUNTING MEDIA*

LIONEL BENJAMIN, B.S., CHICAGO, ILL.

GUM dammar, when in solution with the various well-known solvents, has always been considered an ideal mount-material affording better definition of delicate detail than the commonly used Canada balsam, due to its light colored solution and low refractive index. In spite of these desirable qualities it has been little used for permanent mounts because it forms either macroscopic or microscopic granules which causes confusion when examined at a later date. It has a tendency to become acid, thereby affecting nuclear stains. The solution, upon standing, becomes cloudy and the dried film when deposited from a benzol or xylol solution is hard and brittle. No wonder that this resin has been relegated to the scrap heap.

Gum dammar has been used in the varnish and lacquer industries for many years and while considered a very desirable resin, it presented many problems before it could be used to give a satisfactory film. Varnishes were first made by dissolving the gum in an appropriate solvent and sold to the trade as a cheap substitute. Under these conditions the dried film had a tendency to become white, powdery, and brittle. The films would not withstand shock or friction which made them undesirable.

Chemically, gum dammar has been found to consist of dammarolic acid, alpha dammar resene, and beta dammar resene. The last named constituent being a waxy substance with a low tensile strength is less soluble in the ordinary solvents and has a tendency to crystallize out during the evaporation of the solvent. Many processes have been developed for separating this substance from the other two constituents,† which rely on the difference in solubility between beta dammar and alpha dammar combined with dammarolic acid. The resulting material after the separation has remarkable properties as a varnish gum.

The author has had occasion to prepare many dammar-xylol solutions and has always been confronted with the above difficulties. By taking advantage of the method of treatment used in the lacquer industry, the resulting resin gave very desirable films, had none of the defects found in the raw gum solution, and still retained its excellent optical properties.

*Received for publication. March 2. 1933.

†British Patent No. 309,701; March 15, 1928. German Patent No. 503,615; August 30, 1927.

METHOD OF PREPARATION

A good grade of clear Indian or Malayan dammar is put into solution in the following combination of solvents:

Dammar	450 Grams
Ethyl acetate	57 c.c.
Acetone	57 c.c.
Benzol	266 c.c.
Methyl alcohol	285 c.c.

First mix the solvents thoroughly and then add the gum crushed to about the size of a pea. With occasional shaking during a period of four or five days, the resin will be completely dissolved, leaving all débris and a white slimy stringy mass, the beta dammar, in the bottom of the flask. Allow this to settle and then filter the clear filtrate directly into an evaporating dish. Evaporate on a water-bath until the resin is hard and while still hot add an equal volume of xylol. The addition of the xylol to the hot or melted gum facilitates a solution readily. At this stage sodium bicarbonate in excess is added and the solution is stirred by a mechanical stirrer for a period of twenty-four hours to neutralize any free acidity. The excess sodium bicarbonate is allowed to settle and the clear filtrate is either decanted or poured through a filter. The gum solution is now evaporated on the water-bath to any consistency desired.

In the event that the heating process has darkened the resin solution, decolorizing may be effected by adding activated charcoal to the thin xylol solution, heating for several hours on the water-bath, replacing the evaporated solvent and again filtering.

The advantages of this process are that granules are not formed in the dried film, there is no acidity developed, the solution remains perfectly clear, the optical properties of the gum are not altered, and the resin does not dry too quickly, becoming hard and brittle.

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DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

BILIRUBIN, Concentration and Precipitation of, in the Gall Bladder and Bile Ducts, Elton, N. W., and Deutsch, E. Arch. Path. 15: 818, 1933.

The purpose of this study was to determine whether any physiologic basis existed for the precipitation of bilirubin on preexisting nuclei.

In a study of the concentration of bilirubin in the gallbladders and bile ducts of twenty-three cats and nine dogs the most dilute specimens were obtained, not from the hepatic ducts after cholecystectomy, but from the gallbladders as they were refilling after postprandial evacuation, and the most concentrated specimens were secured from gallbladders after the animals had fasted for from twenty-four to one hundred and forty-four hours. Hepatic duct bile after cholecystectomy invariably yielded a higher bilirubin content and icteric index than the early refillings from the gallbladders.

The factor of bilirubin concentration for dogs was found to be 50.9, for cats, 40.4 and for both animals together 83.3. According to the work of Kalk and Schondube, the factor for either of these animals is appreciably less than that found for man.

The bilirubin icteric index ratio averages lower for gallbladder bile than for bile obtained from the hepatic ducts after cholecystectomy in these animals.

Correlation of the refractive index with the quantitative bilirubin or the color intensity of bile indicates that a critical concentration exists at which bilirubin tends to precipitate out of solution. When this critical concentration is exceeded, the density of bile at once attains its maximum, being constant thereafter in spite of continued concentration. These findings indicate that a limit of crystalloid concentration exists, affecting bilirubin, bile acid derivatives and cholesterol, and effective when the critical concentration is exceeded, and that their further concentration can be only as suspended colloids or undissolved crystals.

Studies on the concentration of bile during fasting showed that the maximum level of bilirubin is usually attained in the gallbladder within twenty-four hours, and that after this interval hepatic bile leaks into the duodenum. The density was found not to increase, even after six days of fasting, above the value attained in twenty-four hours.

The aqueous (direct) van den Bergh reaction on fresh bile simulated any of the various types of reactions described for icteric serums, including immediate blue violet and red reactions, slow and delayed reactions and the negative reaction.

Since gallstones are seldom found in the cat and the dog, the physiologic precipitation of bilirubin during its concentration in the normal gallbladders of these animals cannot be regarded as the exciting cause in the origin of biliary calculi, but affords a physiologic basis for deposition of the pigment on preexisting nuclei.

AGGLUTINATION, H & O following Typhoid Vaccination and in an Unselected Group of Individuals, Dulaney, A. D., Wikle, W. T., Stewart, R. L., Rayfield, J. D., Walker, J. K., and Preacher, A. B. J. Immunol. 24: 229, 1933.

From a study of 90 serums from vaccinated (TAB) individuals and 100 unselected Wassermann serums it is concluded that:

1. Typhoid vaccination stimulates the production of H agglutinins to high titers. O agglutinins are also stimulated, but show much lower titers.

2. O agglutination in serum dilutions of 1:100 was demonstrated in 26 per cent of all vaccinated serums and 11 per cent of a random group of individuals.

3. If O agglutination is to have diagnostic value in typhoid fever, higher dilutions of serum than those recommended by Felix (1:100) must be employed. The authors recommend dilutions of 1:500.

FECES, A Convenient Specimen Container, Frobisher, M. Am. J. Pub. Health 23: 851, 1933.

The outfit consists simply of a 2-ounce, wide-mouth glass bottle with a cork stopper. A wooden tongue blade is cut into halves and one end of each piece is whittled to a sharp, flat point which is thrust firmly into a cut in the narrow end of the cork, the cut in the cork having previously been made with a paring knife. The cork is then fitted into the bottle and the whole sterilized in the hot air oven at 170 C. for two hours.

A good grade of cork or a rubber stopper must be used in order to avoid leakage if pressure develops. If a preserving solution is used, sterilization may be accomplished in steam.

A slightly more expensive modification of this outfit may be made from sheet metal. The wooden paddle is replaced by a piece of metal, and this may be fastened with a few drops of solder (necessitating steam sterilization) to the inside of a metal clamp or screw cover, very much in the same manner as small brushes are supplied in cans of certain brands of automobile "touch up" paint. The possibility of bactericidal or bacteriostatic effects due to the metal must be taken into consideration before adopting such a device. Much will depend on the distance, time and temperatures involved in transporting the material to the laboratory. Both types of paddle can be used more than once. Metal paddles can be made spoon-shaped to facilitate the collection of liquid material.

In collecting the feces specimen the spatula always remains with the bottle, it is not touched by anyone and no disposal or esthetic problem arises.

TUBERCULOSIS: A Technique for Estimating the Number of Tubercle Bacilli in Sputum, Hughes, J. Am. Rev. Tuberc. 28: 279, 1933.

Preparation of Specimen and Smear.—One cubic centimeter of a 10 per cent solution of sodium hydroxide is pipetted into a test tube of 10 c.c. capacity, and 2 c.c. of sputum is added, along with three glass beads. The mouth of the test tube is flamed, and stoppered with a cork. The tube is then placed in a shaking machine in a horizontal position, and agitated. Because of the viscosity of sputum, pieces of ordinary glass tubing, 4 mm. in diameter and 25 cm. in length and graduated by a mark with a file to deliver 2 c.c., have been used instead of the usual type of pipette. Sputum can be readily drawn up into such a pipette by means of a Luer syringe attached to it by rubber tubing.

After being digested and shaken for thirty minutes the specimen is removed from the shaking machine. The following technic is used in making smears:

An area of 400 sq. mm. is marked off on the end of a one-inch microscopic slide by drawing with a diamond pencil a line 1.5 cm. from one end. This measurement introduces an error of less than 0.5 per cent. The slide is then cleansed by being placed overnight in a solution of alcohol, 97 c.c., and acetic acid, 3 c.c. By means of a standard loop, 3 mm. in diameter, made of platinum wire 0.4 mm. in thickness, sold by dealers in laboratory equipment, one loopful of serum used to fix the bacilli to the slide and one loopful of the digested sputum are mixed together on the slide, and are then spread in a uniform smear over the marked-off area of 400 sq. mm. The smear is dried in air, fixed by heat and stained by the usual Ziehl-Neelsen technic. Ten scattered oil-immersion fields are examined and the average number of bacilli per field is estimated. This average is then corrected for the dilution of the sputum by sodium hydroxide by multiplying the number of bacilli seen per field by the factor 1.5.

Since, in the present method, the 3 mm. loop used in making the smear delivers approximately 3.5 c.mm. of treated sputum, it is possible to estimate the number of bacilli in 1 c.mm. of sputum after calculating the average number of bacilli per microscopic field and the number of fields in the smear. This may be expressed by the following equation:

$$\text{Average number of bacilli seen per microscopic field} \times 1.5 \times \frac{\text{Number of microscopic fields of smear}}{3.5} = \text{Number of bacilli in 1 c.mm. of untreated sputum}$$

In the microscope used for this work there were 25,000 fields in the area of the smear, but this figure will vary depending upon the size of the oil-immersion field of different microscopes. It can be readily determined for any microscope by calculating the diameter of the oil-immersion field by focusing on the rulings of a hemocytometer that are 0.05 mm. apart. In doing this it is necessary to use a No. 1 or No. 2 cover slip to cover the rulings, because the oil-immersion lens cannot be used with the heavier cover-slip provided with the hemocytometer.

By working out the equation for estimating the number of bacilli in 1 c.mm. the factor 10,714 was obtained, which, when multiplied by the average number of bacilli per oil-immersion field, gave the number of bacilli in 1 c.mm. of sputum.

RENAL FUNCTION: In Persons With One Kidney, Ellis, L. B., and Weiss, S. Am. J. M. Sc. **186:** 242, 1933.

The renal function of 12 patients who had undergone unilateral nephrectomy was studied by urea and creatinin clearance, concentration and phenolsulphonephthalein tests. Nine of the patients were quite healthy, 2 had pyonephrosis of the remaining kidney and 1 had hypertension.

The 2 cases which were studied immediately after operation showed diminished values for the clearance and concentration tests. The tests on 7 other uncomplicated cases were normal in 2, and one or more of the tests were slightly abnormal in 5. The 3 patients with complications had marked impairment of renal function.

In the absence of complications, the renal function of persons with one kidney is not only adequate, but possesses a reserve capacity as well.

The observations confirm the value of urea and creatinin clearance tests in detecting early and quantitative reductions in glomerular function and of the concentration test as a measure of tubular reabsorptive power.

RENAL FUNCTION: Normal Variations in Renal Function Tests, etc., Ellis, L. B., and Weiss, S. Am. J. M. Sc. **186:** 223, 1933.

On 12 normal subjects urea and creatinin clearance tests were performed and on 5 subjects dilution concentration tests were carried out. These studies were made to show the normal variations of each test and the effect of changes in one test on the others, and to correlate the results with the physiologic functions of the kidney which they measure.

The creatinin clearance test probably gives a close estimate of the absolute degree of glomerular filtration and the urea clearance provides a relative index of this function. Clinically, either test may be used.

The ability of the renal tubules to reabsorb fluid is best measured by a concentration test.

To attain as adequate an estimate as is possible by laboratory methods regarding the state of renal function, the tests mentioned together with an ordinary examination of the urine will suffice.

At best the information gained from these procedures merely supplements that obtained by the actual clinical observation of the patient.

PELLAGRA: A Chemical Peculiarity of Pellagra Blood (Rapid Iodin Decolorization), Campbell, C. H. Am. J. M. Sc. **186:** 266, 1933.

Pellagrous blood reduces iodine solutions at a constantly greater rate than that of any other blood thus far examined. This phenomenon is made the basis of a test which promises to be of clinical value in the diagnosis of pellagra. Many pathologic conditions have not been examined, and it is possible that further work will disclose that other conditions simulating pellagra may give positive reactions. However, the phenomenon of increased rate of iodine decolorization by the erythrocytes in pellagrous blood may lead to fruitful information bearing on the etiology of pellagra, certain entities of avitaminosis and malnutrition.

Reagents.—

Alcohol-ether Mixture: A mixture of 3 volumes of 95 per cent ethyl alcohol and 4 volumes of ether (commercial or anesthetic ether may be used; chemically pure ether has not been tried).

Iodine Solution: Accurately prepared Lugol's solution (5 per cent iodine in 10 per cent potassium iodide).

Color Standards: Prepare a 3 per cent potassium dichromate solution and from this make the following dilutions: 1 to 5, 1 to 10, 1 to 20, 1 to 40, 1 to 80, 1 to 120, 1 to 240. Place these standards in a series of test tubes of the same internal diameter.

Procedure.—

Liquid Petrolatum. Five cubic centimeter samples of normal or nonpellagrous (control) and of suspected pellagrous venous blood are withdrawn and quickly introduced to 15 c.c. centrifuge tubes containing 5 c.c. of liquid petrolatum. The time of collection of each specimen must be recorded. As each sample is collected, the tube is stoppered and shaken very vigorously until the blood is defibrinated. This may best be accomplished by striking the stoppered end of the tube against the palm of the opposite hand. The specimens are then allowed to stand for thirty to forty-five minutes, after which they are again vigorously shaken and centrifuged at 1,000 revolutions per minute for 5 minutes. Before the second shaking it is advisable to remove the stoppers momentarily to permit the entrance of air for adequate oxygenation. When the tubes are removed from the centrifuge three distinct layers may be distinguished, namely, an upper layer of fibrin and oil, a middle layer of serum and a lower layer of erythrocytes, leucocytes, etc., which the author shall designate the erythrocytic layer. If any sample has not been properly defibrinated clots will be present in the lower layer. Such samples should be discarded. One cubic centimeter samples of the erythrocytic layers are now transferred by means of serum pipettes to test tubes of the same diameter as those of the standards. In the removal of these samples from the erythrocytic layer, care must be taken that no oil, fibrin or serum enters the pipette by capillary action as it passes through the two upper layers.

Exactly one hour after taking the venous blood specimen, add to the corresponding erythrocytic sample, slowly and without shaking, 5 c.c. of the alcohol-ether mixture and stopper the tube tightly. After the alcohol-ether mixture has been added to all samples, let them stand for approximately 6 hours at room temperature (25° to 30° C.). At the end of this time add in rapid succession to each tube by means of an accurate micro pipette (Folin micro blood pipette or Kahn serologic pipette) a 0.1 c.c. portion of the iodine solution. Mix gently and replace the stoppers.

BLOOD: A Study of a Lymphocytic Hemogram, Reich, C., and Reich, E. Am. J. M. Sc. 186: 278, 1933.

A lymphocytic hemogram has been studied, using basophilia of the cytoplasm as a criterion of the age of the lymphocytes.

The lymphocytic formula is stable in health, but is more labile than the Schilling index, and cannot be used to gauge the severity of an infection. Its shift to the left in infections nevertheless indicates that the lymphocytes take an active part in combating these conditions.

The lability of the lymphocytic index makes it valuable in detecting minor infections which do not disturb the more stable Schilling index.

The lymphocytic index is superior to the Schilling in guarding the progress of convalescence, and a patient should not be regarded as entirely well until the lymphocytic formula has returned approximately to normal.

Analysis of the experimental data shows that there is a fairly constant lymphocytic formula for normals. For adults this can be expressed as Y forms, 5 per cent; M forms, 50 per cent; O forms, 45 per cent. Variations occur in this formula under different conditions, and we can, therefore, speak of a shift to the left or to the right of the lymphocytic index, depending upon whether the number of younger forms is greater or less than normal

A comparison with the Schilling index in each case shows that the two do not run parallel. The lymphocytic index is not as stable as the Schilling and will shift to the left just as easily in a mild as in an acute infection. Even though the lymphocytic formula cannot be used to gauge the severity of an infection, its shift to the left in these conditions nevertheless indicates that lymphocytes take an active part in combating bacterial invasion. This is seen by the increase in Y and M forms and the decrease in O forms in practically all cases of infection.

The lability of the lymphocytic hemogram makes it valuable in the detection of minor infections which do not disturb the more stable Schilling index. Interesting and confirmatory evidence for using basophilia as a criterion of the age of lymphocytes is seen in the study of the lymphatic leucemias. The marked increase in the proportion of Y and M forms in these cases represents the well-known hyperactivity of the lymphatic system in these diseases. In the myeloid leucemias there is also an increase in lymphocytic activity and a shift of the index to the left.

It is also of interest to note that, according to the lymphocytic index, most patients are not entirely well on being discharged from the hospital and that the period of convalescence is probably much longer than usually expected. A patient should not be regarded as entirely recovered from an infection until the lymphocytic formula has returned to normal.

Technic.—White and differential counts were done for each observation. The total white counts were done with carefully standardized pipettes, and the films were made on slides, care being taken to keep them "rim free." The stain used was Wiseman's Wright-Giemsa. An average of 200 leucocytes was counted and the lymphocytes were divided into three classes: Y (young), M (medium), and O (old) forms, according to the degree of basophilia of their cytoplasm. The Y forms have a deep blue cytoplasm, the M forms moderately blue and the O forms are faintly blue or colorless, and usually show azure granules in the cytoplasm, although the M forms occasionally show them too. A little practice with the above technic will make the three divisions clear. It is necessary, however, that the lymphocytes be examined in those parts of the film in which the red cells do not overlie, as in the thick portions of the film the lymphocytes are likely to overstain. The total number of lymphocytes is noted as well as the number of Y, M and O forms, and the percentages of each are then easily calculated, the procedure being similar to that followed in enumerating the different forms of polymorphonuclears when doing the Schilling index. When the total number of lymphocytes is low it is best to count 300 to 500 white cells to insure more accurate results. The polymorphonuclear leucocytes were tabulated according to the Schilling method in each instance in order to compare the granulocytic and lymphocytic indices.

RENAL FUNCTION: The Fractional Phenolsulphonephthalein Test in Bright's Disease, Chapman, E. M., and Walsted, J. A. Am. J. M. Sc. **186:** 223, 1933.

The fractional method of estimating the elimination of phenolsulphonephthalein (15, 30, 60 and 120 minutes) has been studied in the past two years in 20 normal subjects and a large number of patients with suspected renal disease.

The authors have found that in Bright's disease the fractional test may show evidence of impaired renal function when the test as usually done, with hourly collections, is interpreted as normal.

This fractional test is quite as informative as the urea clearance test and reflects the diminishing function in progressive kidney disease.

Since it is easier to perform, it is the method of choice for routine clinical work.

BLOOD: The Neutrophil in Pernicious Anemia, Heck, F. J., and Watkins, C. H. Am. J. Clin. Path. **3:** 263, 1933.

Fifty cases of pernicious anemia, in all of which there was achlorhydria, have been studied with reference to the lobulation of the neutrophil. Increased segmentation in the neutrophil is the rule, although cases do occur in which there is a left shift and absence of the hypersegmented neutrophil.

In three cases in which there were morphologic changes of pernicious anemia in the presence of adequate quantities of free hydrochloric acid, but in which other characteristic symptoms of pernicious anemia were absent, no essential differences could be found from the former group.

There is no invariably pathognomonic blood picture of pernicious anemia, since the same changes may be demonstrated in sprue and occasionally in an indeterminate group of anemias.

SPIROCHETA PALLIDA, A New Method of Staining (Su Di Un Nuovo Metodo Di Colorazione Del Treponema Pallidum), Pinetti, D. Diagn. E. Techn. Di Lab. 4: 206, 1933.

Pinetti has had excellent results with the modification of Romby's method which is described below:

Reagents.—

A. Acetic Copper Solution:

Chloride of copper	gr. (gm?) 1
Acetate of copper	gr. (gm?) 1
Acetic acid, glacial	15 c.c.

Dissolve the copper salts "as far as possible" in cold distilled water (volume not stated) with frequent shaking and allow to stand 30 minutes. Then heat to boiling and allow to cool, when the acetic acid is added. Filter twice through filter paper and keep in a well-stoppered dark-colored bottle.

If turbidity develops in the solution on standing the clear, emerald-green color can be restored by the addition of a few drops of glacial acetic acid.

B. Fixing Solution:

Osmic acid	gr. (gm?) 1
Distilled water	400 c.c.

C. Staining Solution:

Freshly prepared carbolfuchsin (Ziehl Neelson).

Method.—

1. Cover the smear with a freshly prepared mixture of equal parts of A and B and allow to act for ten minutes. If necessary, add more solution so that the entire surface of the slide remains covered.
2. Pour off the solution and drain.
3. Without washing, cover with the stain and allow to act for five minutes.

In a properly stained slide the treponema are seen as violet red on a rose-colored background. Spirilla, and other organisms are deep red. A deeply stained background indicates an error of technic or in the preparation of the reagents.

Equally good results may be had by using, instead of carbolfuchsin, the stain below:

Victoria blue (Gruhler, R4)	gr. (gm?) 1
Thymol	gr. (gm?) 0.20
Methyl alcohol	30 c.c.
Distilled water	70 c.c.

With this stain, applied as above described, the treponema are an intense blue black, their delicate structure being exceptionally well seen.

The background is clear, precipitate being rare.

REVIEWS

Books and Monographs for Review should be sent direct to the Editor, Dr. Warren T. Vaughan, Professional Building, Richmond, Va.

Red Blood Cell Diameters*

See Editorial in this issue.

Manual of Urology†

THE purpose of this manual is to present the fundamentals of urology so that they may be easily grasped by the student approaching this subject.

The arrangement of the subject matter is designed to emphasize the etiological factors concerning the disease in question in the discussion of which, as the book is intended for the student, only generally recognized conclusions are presented.

To those who are desirous of obtaining a fundamental groundwork in urology upon which to erect a comprehensive knowledge of the subject this book can be recommended as eminently suitable for the purpose.

Blood Pictures‡

THIS small volume, addressed to the practitioner, has for its purpose the presentation of simpler methods of hematological examination, followed by brief résumés of the blood pictures in disease and their interpretation.

The book is succinct in style and serves, as the title indicates, as an introduction to the subject.

This, the third edition, has been revised in keeping with the advances made in this subject.

The Common Cold§

THIS, the Eighth Volume of the Annals of The Pickett-Thomson Research Laboratory, is devoted to a comprehensive discussion of the common cold with special reference to the part played by streptococci, pneumococci, and other organisms.

The publications from this laboratory need no introduction and it is almost unnecessary to say that the present volume is in keeping with and worthy of the standard set by its predecessors.

As is now well recognized, the common cold constitutes one of the most—if not the most—important factors in the morbidity and mortality rates of the civilized world and, because of its economic importance as a cause of physical wastage and inefficiency, has become the subject of concerted and intensive study.

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*Red Blood Cell Diameters. By Cecil Price-Jones M.B. (Lond.) Humphrey Milford, Oxford University Press, New York Branch, 1933. pp. 82, cloth.

†Manual of Urology. By R. M. LeCompte M.D., Professor of Urology, Georgetown University Medical School. Cloth, 317 pages, 46 figures. William Wood & Co., Baltimore.

‡Blood Pictures: An Introduction to Clinical Hematology. By Cecil Price-Jones. Cloth, 72 pages, 5 colored plates. William Wood & Co., Baltimore.

§The Common Cold. By David Thomson and Robert Thomson, The Pickett-Thomson Research Laboratory, St. Paul's Hospital, London. Cloth, pp. 738, 51 plates. The Williams & Wilkins Co., Baltimore.

The present volume, because of its comprehensive review and summary of the work thus far done, ranks as a study of first importance and may well be read by all who are interested in this subject.

Starting with a review of the structure and physiology of the nose and nasal sinuses, the researches concerned with the bacteriological flora of the healthy nose, nasopharynx, mouth, trachea, bronchi, and lung alveoli are next described as determined not only by the investigations of the authors, but also as reported in the literature of the world.

The plurality or nonplurality of colds as a disease, the relationship of the common cold to influenza, and the noncontagious colds are next considered, followed by exhaustive studies of the rôle of various specific organisms as causative agents. Subsequent chapters discuss various predisposing factors, complications, treatment and prevention so that, all in all, the volume is practically encyclopedic in its handling of the subject.

The authors' views deserve careful consideration and can only briefly be summarized here.

They are not convinced that the common cold is a definite single disease but regard it as a group of diseases, arising from varied causes. They recognize, hence, bacterial colds, allergic colds, and possibly virus colds. While not unmindful of the work done concerning the part played by a filter-passing virus as an etiological agent of colds, they believe that to regard all colds as so caused is carrying the theory too far and casting aside without warrant the work proving the important relation of bacteria.

They are convinced that the commonest bacterial variety of cold is the pneumococcal, followed by the streptococcal and streptopneumococcal. Of importance, also, are the colds due to invasion by Pfeiffer's bacillus, and M. catarrhalis, other bacterial colds being infrequent. They comment, also, that it has not yet been shown that filtrable forms of these bacteria may not be the answer to the filter-passing virus theory.

It is impossible, in the space available for review, to convey more than a mere outline of the ground covered by this most comprehensive and valuable report which should be available to all laboratory workers and may well be read with profit by the physician at large.

The format and the excellent microphotographic illustrations will be familiar to those who have seen the previous volumes of this series.

As this reviewer has previously said, it seems a pity that these volumes of such value should not have a more durable covering than paper, for they are sure to be well read and frequently consulted.

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EDITORIAL

Erythrocyte Diameters

FOLLOWING the development of the modern methods in the study and treatment of pernicious anemia, interest in erythrocytology has been widely awakened. Smears that formerly would have been classed as indicative of secondary anemia have occasionally been found to be those of primary anemia. Smears that most pathologists would interpret as characteristic of pernicious anemia have been found to occur in patients that do not have primary anemia. There appears to be a distinct group of this second class. Not infrequently one observes blood smears with clear-cut macrocytosis, with or without anemia, which are definitely not associated with pernicious anemia. As a consequence the measurement of erythrocyte diameters not infrequently becomes a matter of diagnostic laboratory routine.

Attempts to measure red cell diameters are by no means new. While some of the earlier methods were crude and the conclusions were not altogether cor-

rect, it is indeed surprising how accurate they were, considering the methods available. Cecil Price-Jones in his very informative monograph on *Red Blood Cell Diameters* reviews the history of this phase of hemocytometry in conjunction with his presentation of his own very detailed studies of the measurements of red cells in disease and in health.

As long ago as 1673, Leeuwenhoek attempted measurement by the very crude method of placing minute grains of sand in a row, one inch long, all grains being as nearly as possible the same size. By counting the number of grains in contact to make an inch, he was able to estimate the diameter of one grain in terms of fraction of an inch. With this grain for comparison he attempted to measure red cell diameters. Later investigators substituted lycopodium spores with a mean diameter of $\frac{1}{940}$ of an inch for the sand grains.

Jurin in 1718 twisted fibers of silk or hair around a pin, with the successive twists in close contact; counted, under the microscope, the number of coils per unit measure, thereby determining the mean thickness of the hair. A segment of the hair was then compared with erythrocytes. This method was not altogether satisfactory since the hairs and fibers varied in thickness. He calculated the erythrocyte diameters in one series as averaging 7.75 microns, and in another series 12.5 microns.

de Senac in 1749 compared red cell diameters with a standard line, concluding, first that they measured 7.22 microns, later 7.69. Hewson in his famous work on the blood (1770) makes no definite estimate of the size of the erythrocytes but does state that the size varies in different animals and sometimes in the same animal, and again at different periods of life. This was particularly true in the chick.

Prevost and Dumas (1821) employed binocular vision, observing the cells through the microscope with one eye while their image was superimposed upon a scale placed next to the microscope and seen with the other eye, with accommodation relaxed. They estimated the mean diameter as 6.6 micra. Young in 1823 attempted measurement by comparing the luminous halo surrounding the erythrocytes with that surrounding other objects of known diameter, and of approximately the same size, concluding that the diameter of the human red corpuscle was between 5 and 6 microns.

Lister, the father of Lord Lister, and his associate Hodgkin, using Amici's camera lucida (1827) selected 8 microns as the correct size. Gulliver in 1846, using a micrometer eyepiece found the diameter of human red cells to average 7.61 microns. He found that of the elephant, 9.1; the whale 8.03; the musk deer 2.02; the cat 5.6; and dog 7.05.

Price-Jones using a projection apparatus adjusted for a magnification of 1,000 (1910) measured the resultant images with a millimeter scale, recording these figures directly in terms of microns. He found the mean diameter in healthy persons based upon measurement of 10,000 cells to be 7.21 microns, with a mean range from 6.96 to 7.49 (1922). In a later series based upon measurement of 50,000 red cells, the normal diameters were found to range from 4.75 to 9.50 with a mean diameter of 7.202, a standard deviation of 0.487 and a coefficient of variation of 6.325 per cent.

In measuring erythrocyte diameters Price-Jones emphasizes the need for a standard and unvarying technic in preparation. His technic is briefly as follows: A thin spread, air dried, fixed and stained with Jenner stain for two minutes; washing with distilled water and drying, followed by a two-minute super staining with weak aqueous solution of eosin. Deviations from this standard procedure were found to sometimes produce changes in the mean diameters. He prefers counts of 500 cells, with a minimal number compatible with reasonable accuracy of 200. Largest and smallest diameters are taken for each cell, the diameter value of the cell being the average of these two. The arithmetic mean, the standard deviation, and coefficient of variation are then calculated mathematically.[1]

Price-Jones finds, even in normal individuals that there is a distinct diurnal variation in red cell diameters, probably dependent upon the hydrogen ion concentration. There is a gradual increase in size during the day, with a diminution during sleep. The variation may amount sometimes to as much as 0.6 microns. Gentle exercise has no apparent influence but violent exercise increases diameters. Nevertheless rest in bed throughout twenty-four-hour periods does not abolish the diurnal variations. The increase in size following violent exercise may amount to as much as 0.6 microns.

The observation that local cyanosis increases diameters, anywhere up to 0.4 microns, emphasizes the need for obtaining specimens without the use of a tourniquet. Cells in venous blood are larger than those in arterial blood.

These variations in size appear all to be due to changes in hydrogen ion concentration, the swelling being associated with a decrease of alkalinity. It has been shown both in vitro and in vivo that an increase in concentration of lactic acid or of carbon dioxide causes swelling. The washing of CO_2 out of the blood by forced breathing was found to diminish the mean diameter by 0.5 microns. Discontinuance of forced breathing brought a rapid restoration to normal diameters. There is evidence that the blood is more alkaline during sleep than in the daytime.

Pulmonary emphysema, especially when associated with some degree of cyanosis is accompanied by macrocytosis, the mean diameters ranging from 7.33 to 8.17 with a mean diameter for the series of 7.69. On the basis of the same considerations it seems entirely possible that certain chemical poisonings should produce a macrocytic anemia suggestive on microscopic examination of pernicious anemia, but in no way connected with the latter disease. Indeed Beck[2] has described a blood picture following low grade chronic carbon monoxide poisoning, almost indistinguishable from that of pernicious anemia.

The mean diameter following hemorrhage is lower than the mean for healthy persons, averaging 6.879 with a range from 6.3 to 7.225. The distribution curve is shifted to the left of the healthy curve. There is greater variation in the curves, the coefficient of variation averaging 10 per cent, due presumably to the mixture of two types of cells, the mature red cells and smaller, newly formed cells which have been poured into the circulation as a result of sudden overstimulation of the marrow.

In pernicious anemia, Price-Jones found a variation in diameters from 3.75 to 13.0, with a mean diameter of 8.31, over one micron greater than the

average for healthy red cells. The coefficient of variation was as high as 16.3 per cent and the excess of large cells resulted in a megalocytosis of 40.4 per cent. Microcytosis was also present, in about 75 per cent of the cases.

The mean cells of normal persons show a curve of variation which is sharply pointed and quite symmetrical. That for pernicious anemia has a much broader base and is decidedly irregular, usually asymmetrical. This suggests a heterogeneous population of red cells. It seems probable that in the blood of pernicious anemia there are at least three classes of red cells: large cells derived from megaloblasts which have failed to mature due to the lack of the specific intrinsic liver factor; normal size cells from what bone marrow is still reacting normally; and small cells which owe their origin to the same causes which are active in simple acute hemorrhage, that is extra stimulation of bone marrow caused by abnormal destruction of erythrocytes. That these three classes actually exist is further suggested by the observation that the degree of anisocytosis varies directly with the degree of anemia, while the red cell diameters are independent of the degree of the anemia. It would appear that the disease is responsible for the macrocytosis and is associated with the absence of the intrinsic liver factor, but that the presence of microcytes is directly due to bone marrow stimulation resulting from the anemia. Following liver treatment the macrocytosis becomes less pronounced. Indeed in many cases the mean diameter returns to normal.

The mean diameter in microcytic anemia, the chlorotic anemia occurring particularly in women during the child-bearing period and often associated with gastric subacidity or anacidity, was found by Price-Jones to be 6.48 microns with a range from 6.2 to 6.7 microns.

The observations on red cell diameters reviewed above indicate not only the large amount of work which has been done in this field but also the possibilities for future contributions which may be made by those who in their routine work will recognize the apparent slight deviations from normal which will bear further quantitative study and which may lead to a clearer understanding of the subject.

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1. Price-Jones, Cecil: Red Blood Cell Diameters, New York, 1933, Oxford University Press, American Branch.
2. Beck, H. G.: Clinical Manifestations of Carbon Monoxide Poisoning, Ann. Clin. Med. 5: 1088, 1927.

—*W. T. V.*

Erratum

In the article by Drs. Macht and Paulson *Phytopharmacology of Stomach Washings in Various Digestive Disorders and Pernicious Anemia* published in the November, 1933, issue, page 164, line 32, the percentage should read 86 instead of 6.